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Editorial

Precision Rehabilitation 2.0: From One-Size-Fits-All Therapy to Intelligent Personalized Care

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Rehabilitation sciences are in a transition mode where generic treatment models are more and more being replaced by personalized treatment models using technology. Since there are substantial differences in recovery potential, biomechanics, psychosocial status and lifestyle amongst patients, the interventions for rehabilitation have, to a great extent, been standardized in the past 30 years. The increasing integration of Artificial Intelligence (AI), wearable technologies, digital biomarkers, robotics, and the use of big data analytics, however, is changing rehabilitation to a more individualized and adaptive field, called precision rehabilitation[1].

Precision rehabilitation is not simply modifying exercise intensity or frequency according to the patient's tolerance, but maximizing the approach. It is an integrated and evidence-driven treatment model, with ongoing monitoring, predictive analytics and person-specific functional profiling to guide treatment decisions.

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Precision rehabilitation, like precision medicine, aims to optimize recovery by using patient-specific physical, behavioral, cognitive, and environmental factors[2].

AI-based systems and wearable sensors have been contributing to this paradigm shift in recent times. Smart wearable devices are now capable of continual monitoring of gait, posture, range of motion, muscle activity, and cardiovascular responses across the real world. For neurological rehabilitation, wearable systems equipped with machine learning algorithms are gaining popularity to evaluate motor recovery, to forecast functional outcomes, and to adapt rehabilitation exercises based on patient performance[3]. The concept of precision rehabilitation has been further reinforced by AI, which allows clinical decision-making to be adaptive. AI-driven rehab platforms can track patient progress in real-time, detect patterns that human healthcare providers cannot, and provide personalized interventions. Additionally, AI-driven rehabilitation aids can help improve adherence, treatment precision, and outcomes, with remote monitoring and tele-rehabilitation models becoming more prevalent, and robotic rehabilitation devices and virtual reality platforms providing increasingly personalized rehabilitation sessions based on the patient's motor functions, cognition, and rehabilitation objectives[4].

One other exciting opportunity for precision rehabilitation is “digital twins” and predictive

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recovery models. These systems can simulate the outcomes of rehabilitation using biomechanical, physiological and behavioral data to create virtual representations of patients to optimize treatment. Innovation like this could one day enable doctors to foresee the course of recovery and choose the most effective treatments to improve their chances of success more than ever before[5].

Although promising, there are several hurdles to overcome in order to make precision rehabilitation routine clinical practice. However, high costs, weak technological capabilities, lack of clinician training and data privacy, and algorithmic bias remain as obstacles to broad deployment, especially in low and middle-income countries. Furthermore, too much dependence on technology should not distract from the role of the human touch, empathy, and clinical reasoning in rehabilitation practice.

However, precision rehabilitation is the direction of allied health in the future. The use of intelligent technologies in patient-centred care can help to enhance the effectiveness, accessibility, and responsiveness of rehabilitation. The next generation of rehabilitation practice will be shaped by the ability of rehabilitation professionals to move forward in this rapidly changing environment, innovate using data, while at the same time maintaining the humanistic elements of care.

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Original Article

Evaluation of Physical and Mechanical Properties of Gelatin-Pectin Films Reinforced with Banana Peel Extract for Improved Food Packaging Material

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ABSTRACT

Background: Plastics are a rapidly increasing component of municipal solid waste as they do not decompose easily and thus causes an adverse effect to the surroundings. To overcome this, the use of bioplastics as packaging material particularly in the food industry is increasing. Currently, an innovative, eco-compatible and cost-effective protocol has been developed to synthesize a bio-based composite film using gelatin (G) loaded with pectin (P) and banana peel extract (BPE), and it was subsequently assessed for its physical and mechanical properties.

Methods: The casting solution procedure was used to produce the biodegradable food packaging films with different blends: G, G + P and G + P + BPE. Glycerol was used as a plasticizer. The physical tests included thickness, film moisture, swelling index and solubility. The mechanical tests were elongation at break, young's modulus and tensile strength.

Results: The final G + P + BPE composite film became thicker (0.145 mm as compared to 0.084 mm). The moisture content increased to 26.19 % while the solubility in water decreased (95.11% to 55.77% respectively) and swelling index lowered to 53.21% from 67.66%. The elongation at break was suppressed to 8.215%, however the young's modulus and tensile strength appeared high 74.52 MPa and 14.87 N/mm2 respectively.

Conclusion: The acquired results has shown the blended film has the potential to be used as a bioactive edible packaging material to maintain the viability of packaged food.

Keywords: *Edible composite films, Food packaging, Gelatin + Pectin-based films, Mechanical properties, Physical properties*

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INTRODUCTION

The petroleum-based polymers pose a significant hazard to fossil fuel resources and surroundings. Plastics are a rapidly increasing component of municipal solid waste, also a significant part of one-time-use items such as PET bottles, bags and wrappings of products. They do not decompose easily, leaving a negative impact to the land, water ways and air. Owing to this there has been an increased push to adopt the use of bio-plastics as packaging[1-2]. Innovative biopolymer materials have become highly utilized in food and pharmaceutical sectors due to their broad approval to the consumers. Interestingly, bioplastics have also been established to be useful in their bio-medical benefits and explored to be used in wounds, surgical dressing and contribute to decreasing the use of fossil fuel and sustainability[3-4].

Gelatin (G) is a polypeptide polymer with a high concentration of glycine, proline and hydroxyproline. Gelatin is extracted from skin and bones from different animals including cows, goats, camels, fishes and insects[5]. Due to its excellent biodegradability and biocompatibility,

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used as structural material in food packaging, pharmaceuticals, tissue engineering, etc. as the thermally denatured product of collagen. It becomes more stable by blending it with different biopolymers and nanoparticles to boost the strength and barrier performance[6]. It has a range of unique characteristics such as the capacity to gel, thicken, stabilize, and emulsify. It is healthy, enhances shelf life and gives bioplastics good tensile strength and elasticity. Gelatins have high potency as biodegradable plastic for packaging film[7]. Although polysaccharides like chitosan, cellulose, starch, etc. are well studied as decomposable packaging materials, exploitation of pectin (P) has not been fully harnessed. It is normally obtained from citrus (orange and lemon) peels. It contains an α-1–4 linked D-galacturonic acid (GalA) polysaccharide backbone. It is inexpensive, has accessibility, biodegradable, renewable, biocompatible, acts as a film forming material and gelling agent. It finds various uses in food, packaging, pharmacy, biomedicine and agriculture. Moreover, it functions as an oxygen and oil barrier, harmless with notable mechanical strength. It is a good candidate material to develop in the future[8-9]. Musa acuminata is a banana species which is indigenous to South Asia and is a member of the Musaceae family[10]. In Pakistan it is produced in tonnes and mostly cultivated in Sindh province[11]. Banana fruits outlived 10 to 12 months after planting. The tree is cut after harvesting and new growth of the tree is obtained by the stem and rhizome wasting production of rejected tonnes of fruits. The enormous waste turns into a useful resource for a number of different sectors such as in biofertilizers, biogas, pharmacy, food fortification, bioplastics, etc[12]. According to one study, adding more banana peel powder to PLA composite films enhanced the rate of biodegradation[13]. Agro-waste from banana processing advances nanotechnology, replenishable energy and the creation of biodegradable plastics for a circular economy. This innovative, cost-effective, and environmentally friendly solution relieves strain on the world economy[14].

Therefore, the aim of this study is to prepare the Gelatin and Pectin films incorporated with banana peels extract and also evaluated their mechanical and physical properties for the effective food packaging material. These films would enhance food safety, quality and improves the shelf-life of food products.

METHODOLOGY

Materials

Banana peels were obtained from nursery of PCSIR, Karachi city. Copper sulphate (CuSO4) and glycerol (GLY) were purchased from Sigma Aldrich, Germany. Gelatin (isolated from piscine skin) was procured from Rossmoor Food Products, Karachi, Pakistan. Pectin from citrus fruits (79 % galacturonic acid and 8 % methoxyl content) was procured from Bin Dawood Flavours Pvt Ltd. The chemicals utilized were of analytical-grade and required no further refinement.

Isolation of Banana Peels Extract (BPE)

The peels were washed with running water and oven dried at 60℃ for a day. The peels were pulverized into a fine powder and kept in non-transparent plastic jars at room temperature. Aqueous extraction was done by adding 5 g powder to 50 mL of deionized water, letting it stand for a night and filtered through standard Grade 1 filter paper. The isolated extract was then refrigerated at 4℃ to be used further[15].

Fabrication of Gelatin-Pectin with BPE Composite Films

The casting solution procedure with slight modifications was used to produce the biodegradable food packaging films[9]. The formulation of biopolymer films with their compositions is shown in Table 1. Briefly, for control, 2 g of gelatin powder (G) was solubilized in 30 mL of diH2O and mixed for half an hour at low heat. Next, 0.5 mL of glycerol, a plasticizing agent was mixed with the aid of a magnetic stirrer at 60 ℃ up to the point of complete gelatinization. Also, 0.5 g of pectin (P) powder was added to 30 mL diH2O and stirred for 20 min. The magnetic stirrer was used to mix both solutions to produce a G + P composite film. Additionally, 1 mL BPE was incorporated to the blend above (G + P + BPE). The solutions were stirred continuously for half an hour and cast onto glass plates individually. The films were dried for two days at room temperature, then removed from the plates and kept in an air-tight drying vessel for further analysis.

Table 1: Formulation of biopolymer films with their compositions

Film formulations	Gelatin (g)	Pectin (g)	Banana peel waste extract (mL)	Glycerol (mL)
Gelatin	2	-	-	0.5
Gelatin + Pectin	1.5	0.5	-	0.5
Gelatin + Pectin + Banana peel extract	1.5	0.5	1	0.5

Physical Properties of BPE Composite Films

Film thickness

Each film thickness was measured through a dial micrometer (Mitutoyo Japan Vernier Caliper 6), ranging from 1-1000 mm. For best precision, four positions on the film were picked randomly and the average values for thickness were considered.

Film moisture

The original weight of the film (3 x 3 cm2) was noted, left in an oven at 110 ℃ for 24 h and final weight was noted[16]. FM (%) was obtained through the following formula:

$$\mathrm{FM}\ (\%) = [(\mathrm{Wf} - \mathrm{Wi})/\mathrm{Wf}] \times 100$$

Film solubility

The FS was ascertained by the approach, with slight adjustments[17]. The film's (3 x 3 cm2) original weight was noted, followed by drying at 105 ℃ for half an hour. After this, for 24 h they were immersed in 50 mL dH2O at room temperature. After incubation period, the insoluble fraction was removed, dried at 105 ℃ again for another 24 h and the final weight was calculated. Each film was estimated to find its solubility:

$$\mathrm{FS}\ (\%) = [(\mathrm{Wi} - \mathrm{Wf})/\mathrm{Wi}] \times 100$$

Swelling Index (SI)

The SI of the prepared films has been experimented with minor modifications[18]. The biofilms (3 x 3 cm2) weight was noted and placed in 200 mL dH2O for 30 min. Excess water was blotted off using a filter paper and weighed again. The calculation of the SI (%) was carried out with help of the given formula:

$$\mathrm{SI}\ (\%) = [(\mathrm{Wf} - \mathrm{Wi})/\mathrm{Wf}] \times 100$$

Mechanical Properties of BPE Composite Films

Calculations of the structural characteristics including Young's Modulus, Elongation at break and tensile strength were determined by a texture analyzer (Model 4301, INSTRON Lloyd Instrument Ltd., Fareham, UK) as defined in the procedure of ASTM 828-97. In particular, 10 x 1 cm2 sized strips of each film specimen were firmly attached in the grips of the analyzer to be tested. The probe was operated at 1,5 and 10 mm/s speed for the conditioning, evaluation and concluding stages, respectively. The probe was operated at the length of 100 mm and an initial force of 5g, while a 10g of load cell was attached to the probe[19].

Statistical Analysis

The statistical data were evaluated using Origin 9.4 software (Origin Lab Corporation, Northampton, MA 01,060 USA) and IBM SPSS software (SPSS 26.0 for windows, SPSS Inc., Chicago, IL, USA). Each of the statistical data sets was measured three times and presented by the mean value ± standard deviation. Comparison of the recorded data showing significant variations was carried out based on the One-way Analysis of Variance (ANOVA). The level of significance was however established as $p \leq 0.05$.

RESULTS

Physical Properties of the Films

Film thickness

The composite films thickness was recorded with the help of a micrometer at four random points. The results in Table 2 shows the control (G) exhibited the thinnest film (0.084 ± 0.001 mm) while thickness increased with adding pectin (G + P = 0.114 ± 0.001 mm) and banana peel extract (G + P + BPE = 0.145 ± 0.001 mm).

Film moisture

The moisture findings in Table 2 increased progressively with formulation modification. The control gelatin exhibited the lowest moisture content (G = 15.95 ± 0.2 %) while the addition of pectin (G + P) and banana peel extract in (G + P + BPE) significantly increased the film moisture to 22.49 ± 0.01 % and 26.19 ± 0.01 % respectively.

Table 2: Physical characteristics of the formulated food packaging films.

Film formulations	Thickness (mm)	Film moisture (%)	Swelling index (%)
Gelatin	0.084 ± 0.001	15.95 ± 0.2	67.67 ± 0.7
Gelatin + Pectin	0.114 ± 0.001	22.49 ± 0.01	74.64 ± 0.4
Gelatin + Pectin + Banana peel extract	0.145 ± 0.001	26.19 ± 0.01	53.21 ± 0.3

The mean ± standard deviation values of the developed Films. P<0.05

Swelling Index (SI)

The results in Table 2 of swelling index varied with composition. The control (G) exhibited SI of 67.67 ± 0.7 %, which increased to 74.64 ± 0.4 % with the incorporation of pectin (G + P). However, the addition of banana peel extract (G + P + BPE) reduced the SI to 53.21 ± 0.3 %.

Film solubility

Fig. (1) shows the solubility % of three different film compositions (G, G + P, G + P + BPE) in water solvent. Pure gelatin has the highest solubility of 95.45 %. However, with the addition of pectin and banana peel extract decreased the solubility to 79.18 % to 55.77 % respectively.

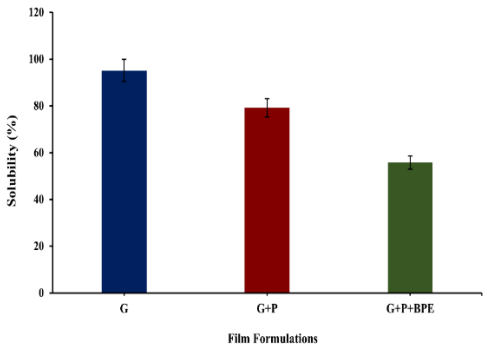

Fig.1. Solubility of the formulated food packaging films in solvents. G (Gelatin), G + P (Gelatin + Pectin) & G + P + BPE (Gelatin + Pectin + Banana peel extract)

Mechanical Properties of Films

Tensile strength (TS)

The tensile strength values in Table 3 show that the film's mechanical properties are greatly dominated by the composition. Control gelatin (G) has the lowest tensile strength (3.829 ± 2.37 N/mm2) but elevated to 12.455 ± 1.11 N/mm2 when pectin (G + P) was added and further boosted to 14.87 ± 5.77 N/mm2 with the inclusion of banana peel extract in G + P + BPE.

Table 3: Mechanical characteristic of the formulated food packaging films

Film formulations	Tensile strength (N/mm^2)
Gelatin	3.829 ± 2.37
Gelatin + Pectin	12.455 ± 1.11
Gelatin + Pectin + Banana peel extract	14.87 ± 5.77

The mean ± standard deviation values of the developed Films. P<0.05

Young's modulus (YM)

The Young's modulus of the bioplastic films increased progressively with compositional modifications. The control (G) in Fig. (2) has the minimum YM value of 15.32 MPa but the addition of pectin (G + P) significantly elevated to 39.85 MPa. A further pronounced increase was observed for G + P + BPE composite showing the highest value of 74.523 MPa, demonstrating improved rigidity and mechanical strength of films.

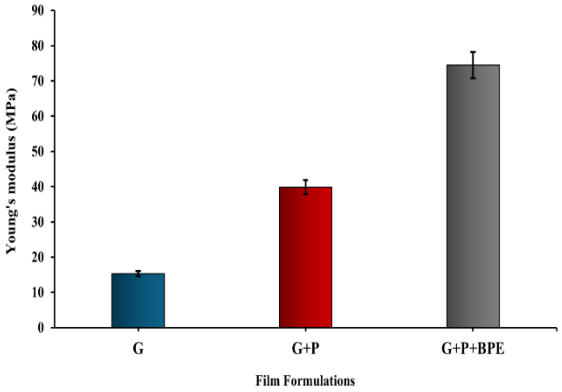

Fig.2. Young's modulus of the formulated food packaging films.

Elongation at break (EAB)

Flexibility and stretchability are signified in elongation at break. In Fig. (3), the control (G) exhibited the highest EAB value of 72.65 % and when it is combined with pectin (G + P) the elongation reduced to 41.85 %. A further decline was observed with the addition of the banana peel extract (BPE) to the mixture of G + P + BPE at the value of 8.215 %, reflecting reduced ductility and enhanced rigidity of the films.

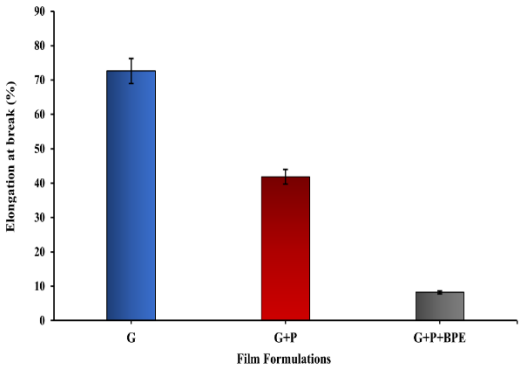

Fig.3. Elongation at break of the developed food packaging films.

DISCUSSION

The gelatin-control (G) is the thinnest film because it has low solids content, the ones which contain pectin and banana peel extract (G + P + BPE) are thicker because the total solids and viscosity progressively increased. Banana peel extract contains high levels of polyphenols, starch, cellulose fibers, organic acids, sugars and minerals that interlink with gelatin and pectin to form cross-linkage, hydrogen-bonded network as well as some covalent bonds to form a stronger and more stable film. Correspondingly, banana peel-loquat leaf extract films[19] and G + P-lemon grass essential oil film[9] exhibited indistinguishable results.

The increase of moisture content is attributed to the hydrophilic nature of pectin due to the contribution of polar groups by pectin[9], and the polysaccharides in BPE further elevates the solid content and polymer interactions due to presence of fibers, phenols and other bioactive compounds that binds G and P. A similar observation was detected in another study about smart banana starch film (BSF) blended with banana peel extract[20-21.] The (G) and (G + P) have the highest swelling because of their high hydrophilic properties and usage of numerous polar groups[22]. The incorporation of (BPE) reduced the swelling index, because the polyphenols and tannins in BPE form extra interactions with gelatin and pectin chains to form a denser, non-permeable matrix to water[23]. All-in-all, G + P + BPE films exhibit the minimal swelling and the highest structural stability at aqueous conditions. Solubility is highest in gelatin due to their hydrophilic chains, but decreased with incorporation of pectin due to the formation hydrogen bonds with gelatin. The banana peel extract further decreased due to additional cross-linking, which improves the compactness of the networks their hydrophobicity, and dissolution resistance[9,21].

The gelatin (G) has lowest tensile strength since the polymer chains are rather weak and flexible. The tensile strength enhanced with addition of pectin in (G + P) by reinforcing the network through hydrogen bonding[9], while banana peel extract in (G + P + BPE) further boosted the film via phenolic compounds promoting cross-linking within the matrix[24] resulting in highest tensile strength. The control (G) has the lowest Young's modulus since it contains loose and flexible protein chains resulting in low stiffness. Inclusion of pectin (G + P) has a strong effect on stiffness as it forms strong hydrogen bonds with gelatin, enhance crosslinking and restrain the mobility of the molecules. BPE addition to G + P + BPE formulation further enhances the stiffness due to polyphenol-induced cross-linking forming a compact and rigid structure[24]. The decrease of elongation at break with the addition of pectin and banana peel extract indicates a substantial loss in ductility. The control (G) possesses good gelatin elongation which is the natural flexibility and when it is combined with pectin (G + P) the elongation reduces because the combination of the two forms a stronger net-work[9]. The addition of the banana peel extract to G + P + BPE slows down the process because the interactions (hydrogen and cross linking) between the polymer networks hinder the movement of the G and P chains resulting in a stronger, but less ductile film[20].

CONCLUSION

To conclude, biodegradable G + P + BPE films were developed successfully. The solution casting technique was used to determine mechanical and physical characteristics of bioplastic film at the mixture of gelatin, pectin matrix and banana peel extract (BPE). When compared to the G + P film, the moisture level of the ultimate blend G + P + BPE film were enhanced whereas the swelling index and the solubility were reduced. The Young's modulus

and tensile strength were increased while elongation at break was reduced. The hybrid film is likely to be used as an enhanced food packaging film to increase the shelf life of sealed food.

Author Contributions

NW: Data Collection, Data Analysis and interpretation, Manuscript Writing and Final Approval.
FI: Conception & design, Data Analysis and interpretation, and Critical Reviewed.
II: Data Collection, Data Analysis and interpretation, and Final Approval.
MAA: Conception & design, Data Analysis and interpretation, Critical Reviewed and Final Approval.

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No conflict of interests.

Data Availability
Data will be available on request

Ethics approval
Not applicable

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Original Article

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Effects of Periodized Circuit Training Versus Conventional Strength Training on Cardio metabolic and Pulmonary Outcomes in Type 2 Diabetes Mellitus: A Randomized Controlled Trial

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is associated with poor cardiovascular and metabolic outcomes. Although periodized circuit training (PCT) and conventional strength training (CST) are both recommended in the management of T2DM, the relative effectiveness of each training method across these multidimensional outcomes has not been clearly defined. Therefore, this study aimed to determine the effects of a 12-week PCT program with CST on pulmonary function, cardiovascular fitness, glycemic control, and Health related Quality of Life (HRQoL) in adults with T2DM.

Methods: Fifty adults with T2DM were randomly allocated to a PCT group (Group A, n=25) or a CST group (Group B, n=25) in a single-blind randomized controlled trial conducted at JPMC Hospital, Karachi. Both groups performed home-based training 3 times/week for 45-minute over 12 weeks. The baseline and post-intervention outcomes included FVC, FEV1, FEV1/FVC ratio, PEFR, six-minute walk test (6MWT) distance, HRQoL (SF-36), and HbA1c. Wilcoxon Signed-Rank and Mann–Whitney U were used for analysis.

Results: Both groups demonstrated important within-group improvement of most outcomes ($p < 0.05$). Between group analysis showed that there were no significant differences in FVC, FEV1, PEFR, 6MWT, HRQoL or HbA1c. Nevertheless, the FEV1/FVC ratio increased significantly in the PCT group compared to the CST group (83.52 ± 3.42% vs. 81.52 ± 2.85%; U=202.5, p=0.032), which is indicative of better airway efficiency.

Conclusion: Both PCT and CST are effective home-based interventions to improve pulmonary function, cardiovascular fitness, glycemic control and quality of life in T2DM patients. PCT demonstrated greater improvements in airway efficiency, suggesting broader pulmonary benefits.

Keywords: *Exercise Therapy, Glycated Hemoglobin A, Pulmonary Function Tests, Resistance Training, Type 2 Diabetes Mellitus*

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INTRODUCTION

Resistance of cell to take up insulin and relative deficiency of insulin are the hallmarks of type 2 diabetes (T2DM), a metabolic disease[1-2]. Long-term glucose elevation brought on by the illness has a significant risk of multiple system complications[3]. Seventy percent of patients with insulin independent diabetes died from cardiac and vascular disease, and new research indicates that pulmonary dysfunction, including forced expiratory volume (FEV1), forced vital capacity (FVC), and diffusion capacity, are additional clinically underappreciated consequences[4]. When compared to comparable non-diabetic populations, complications from diabetes are linked to lower quality of life related to health-as well as decreased physical and psychological well-being[5].

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The American Diabetes Association, also known as the ADA, states that physical activity (APA) is the cornerstone of T2DM management[6]. People with insulin independent diabetes mellitus should also engage in resistance training twice to thrice a week to obtain at a minimum of 150 minutes moderate-intensity aerobic activity per week[7]. By increasing GLUT4 translocation in skeletal muscles, reducing visceral fat, and releasing myokines with anti-inflammatory qualities, exercise improves glycemic control[8]. In individuals with T2DM, structured exercise interventions have been demonstrated to lower glycated hemoglobin (HbA1c) levels by 0.6–0.7%, lower systolic blood pressure, and improve lipid levels[9]. We are unsure of the exercise modality that has the biggest overall impact on addressing the various cardiometabolic, pulmonary, and quality of life deficits associated with having T2DM, despite a strong collection of evidence[10].

Traditional ST (Strength Training) has been scientifically proven to have positive effects on overall muscular, bone mineral density, and insulin sensitivity, however, the lack of a strong cardiovascular component limits the effectiveness of the traditional ST system on cardiorespiratory fitness and pulmonary function[11]. Circuit training (CT) combines both Strengthen and aerobic training by performing both modalities sequentially at different stations[12]. When properly programmed with periodized variations in intensity, volume, and exercise selections, CT has the potential to provide multiple physiological adaptations while decreasing the chances of overtraining and plateaus of performance[13]. Therefore, when using the periodized CT (PCT) method, it may have the opportunity to provide more extensive physical benefits than either CST or CT alone with regard to the complex physiological issues associated with T2DM[14].

Despite the theory that PCT has multiple advantages over CST, there are limited studies that have conducted direct comparisons of these two ST methods on T2DM populations[15]. Most importantly, there is a lack of research on the differences between PCT and CST in reference to pulmonary outcomes, cardiorespiratory fitness, glycemic control, and HRQoL (Health Related Quality of Life) through the conduct of randomized controlled studies. This issue is of particular concern in limited resource clinical settings in which the prescription of exercise needs to be both efficient and effective. As a result, the objective of this randomized controlled trial is based on comparing the effects of periodized circuit training and conventional strength training on cardiovascular fitness, pulmonary function, level of glucose in blood, and health-related quality of life in adults having insulin independent diabetes mellitus in order to develop accessible, evidence-based exercise regimens for this group.

METHODOLOGY

Study Design and Setting

This randomized clinical trials with control groups were performed at JPMC Hospital, Karachi. The study was registered at ClinicalTrials.gov (Identifier: NCT07484672) and received ethical approval from Riphah International University (Protocol Record No. REC/RCR&AHS/24/0388). Data were collected from May 2025 to November 2025.

Sample Size

A sample size of n=42 participants was calculated using Epitool analysis software with 80% power and 95% confidence interval. Taking a value of 6-minute walk test at baseline (M0) 138±21 and after six months 137± 54 (M6)[16] from a reference study an aforementioned sample size was estimated. Following the addition of a 20% attrition rate, the final sample comprised 25 participants per group (total n = 50).

Selection Criteria

Participants of both genders aged group between 40 to 70 yrs, with a documented diagnosis of insulin independent diabetes mellitus with at least one year, HbA1c less than 10%, who were hemodynamically stable, insulin-independent, non-smokers, and without orthopedic limitations or gait and balance impairments were recruited. Individuals with uncontrolled blood pressure, severe cardiac disease, history of cardiac surgery, neurological or systemic comorbidities, pregnancy or lactation, or those engaged in any structured physical activity program within the preceding six months were excluded.

Data Collection Procedure

Data collection begins followed by ethical approval and ClinicalTrials.gov registration. Before enrollment in the Study model handwritten informed consent was received from all the subjects. Participants were screened priorly for eligibility and then using lottery method the two

groups were randomly allocated. The study employed single-blind design, with the assessor physiotherapist blinded to group allocation. Baseline assessments included blood glucose monitoring, pulse oximetry, and sphygmomanometery.

Group A performed Periodized Circuit Training (PCT) comprising a session based on 45-minute, thrice a week, including a warm up session of 5-minute, aerobic exercise like (walking and jogging), session based on 20 minutes 15 minutes of resistance exercise (bodyweight and scissor exercises), and cool-down for 5-minute. Group B performed Conventional Strength Training (CST) comprising a sessions 45-minute, thrice a week, including a 5-minute warm-up, 35 minutes of strength and endurance training (of glute bridge, prone back extension, and ankle plantar flexion), and a 5-minute cool-down. Both interventions were home-based and conducted at moderate intensity.

Outcome Measures

All evaluation were conducted at baseline and after 3 months (approximately 12 weeks) of intervention.

i. **6-Minute Walk Test (6MWT)**
Cardiovascular fitness was assessed using the 6MWT, a submaximal exercise test measuring the maximum distance walked in six minutes along a flat 30-meter corridor[17]. Demonstrating excellent reliability and validity concerning functional capacity assessment, walk test in the window of 6-minute is also recommended by the American Thoracic Society guidelines for use in individuals with insulin independent diabetes mellitus (T2DM).

Spirometry assessment of pulmonary function was carried out using a calibrated digital spirometer adhering to the ATS/ERS standardized methods of measurement.

Forced Expiratory Volume in one second (FEV1), Forced Vital Capacity (FVC), FEV1/FVC ratio, and Peak Expiratory Flow Rate (PEFR) were recorded. Each participant performed a minimum of three acceptable maneuvers in a seated upright position, with the best effort recorded[18].

ii. **Health-Related Quality of Life (HRQoL)**
The assessment of Quality of life was obtained by using the SF-36 Health Survey, a validated 36-item self-reported questionnaire encompassing eight domains of physical and psychological well-being, each scored from 0 to 100, with higher scores reflecting better quality of life[19].

iii. **Glycemic Control**
HbA1c serves as the standardized marker for controlling blood glucose level for long period in T2DM management[20]. The values were taken at baseline and after twelve weeks of intervention to determine the effects of intervention on glycated hemoglobin levels.

Statistical Analysis

SPSS version 26 was use for data calculation and analysis. A level of significance was set at $p < 0.05$. The determination of data Normality was obtained by using a Kolmogorov-Smirnov test. Between group analyses was performed using a Mann-Whitney U test and within group analyses was performed using a Wilcoxon Signed-Rank Test. For representing demography of the recruited participants descriptive statistics was run and represented in the form of frequency and percentages.

Demographic Characteristics

A total of n=50 participants were enrolled and equally distributed between two groups (Group A with (Periodized Circuit Training, n = 25) and Group B with (Conventional Strength Training, n = 25). The overall mean age of participants was 54.66 ± 8.89 years, indicating that the sample predominantly comprised middle-aged to older adults. The mean BMI was 24.65 ± 4.90 kg/m² (table 1). Among the groups the participants, 32 (64%) were classified as overweight (BMI 25.0–29.9), 11 (22%) as obese (BMI ≥ 30.0), and 7 (14%) as having a healthy weight (BMI 18.5–24.9). There was equal gender distribution among the groups, with 25 males (50%) and 25 females (50%) across the total sample.

Table 1: Demographic Characteristics of Participants

Variable	N	Mean ± SD
Age (years)	50	54.66 ± 8.895
BMI (kg/m²)	50	24.65 ± 4.90
Frequency as per BMI Categories		

Category	Frequency	Percentage
Normal	7	14
Overweight	32	64
Obese	11	22
Frequency Gender wise distribution		
Male	25	50
Female	25	50

Normality Testing

The Shapiro-Wilk test confirmed that all baseline variables, including age, BMI, FEV1, FVC, FEV1/FVC, PEFR, HRQoL, HbA1c, 6MWT, heart rate, and SpO_2, were non-normally distributed (all $p < 0.05$). Accordingly, non-parametric statistical tests were applied for all subsequent analyses.

Effects of intervention after 12 weeks of treatment

Prior to treatment Mann-Whitney U test confirmed no statistical significance between-groups, the difference at baseline score across all outcome measures thus ensuring comparability of both groups.

In Group A there were statistically significant within-group improvement to nearly all outcomes associated with an intervention over 12 weeks. FEV1 improved from 2.32 ± 0.20 L to 2.59 ± 0.157 L ($Z = -3.970$, $p < 0.001$), the FEV1/FVC ratio increased from 79.56 ± 1.19 to 83.52 ± 3.42 ($Z = -3.937$, $p < 0.001$), and PEFR improved from 384.40 ± 21.80 to 402.80 ± 21.50 L/s ($Z = -4.367$, $p < 0.001$). FVC showed a non-significant increase from 2.96 ± 0.232 L to 3.09 ± 0.184 L ($Z = -1.859$, $p = 0.063$). Functional capacity measured by the 6MWT improved significantly from 539.20 ± 17.05 m to 593.20 ± 11.80 m ($Z = -4.425$, $p < 0.001$). HRQoL scores increased from 72.24 ± 1.62 to 79.20 ± 1.65 ($Z = -4.415$, $p < 0.001$), and HbA1c decreased significantly from 7.34 ± 0.14% to 6.70 ± 0.09% ($Z = -4.397$, $p < 0.001$).

Group B also had statistically significant improvements within group across all measures of functional ability). FVC improved from 2.87 ± 0.23 L to 3.13 ± 0.20 L ($Z = -3.735$, $p < 0.001$), FEV1 from 2.34 ± 0.185 L to 2.62 ± 0.14 L ($Z = -3.786$, $p < 0.001$), PEFR from 386.40 ± 19.97 to 406.80 ± 20.35 L/s ($Z = -4.449$, $p < 0.001$), and FEV1/FVC ratio from 79.56 ± 1.04 to 81.52 ± 2.85 ($Z = 2.369$, $p = 0.018$). The 6MWT distance improved from 541.20 ± 15.08 m to 596.40 ± 10.75 m ($Z = 4.432$, $p < 0.001$). HRQoL scores rose from 72.16 ± 1.40 to 78.84 ± 1.54 ($Z = 4.412$, $p < 0.001$), and HbA1c declined from 7.404 ± 0.124% to 6.74 ± 0.076% ($Z = 4.396$, $p < 0.001$). (Table 2)

Table 2: Effects of Intervention after 12 weeks of treatment

Outcome Measure	Group	Pre Mean ± SD	Post Mean ± SD	Z	p-value
FVC (L)	A	2.96 ± 0.232	3.09 ± 0.184	−1.859	0.063
	B	2.87 ± 0.23	3.13 ± 0.20	−3.735	<0.001
FEV1 (L)	A	2.32 ± 0.20	2.59 ± 0.157	−3.970	<0.001
	B	2.34 ± 0.185	2.62 ± 0.14	−3.786	<0.001
FEV1/FVC (%)	A	79.56 ± 1.19	83.52 ± 3.42	−3.937	<0.001
	B	79.56 ± 1.04	81.52 ± 2.85	2.369	0.018
PEFR (L/s)	A	384.40 ± 21.80	402.80 ± 21.50	−4.367	<0.001
	B	386.40 ± 19.97	406.80 ± 20.35	−4.449	<0.001
6MWT (m)	A	539.20 ± 17.05	593.20 ± 11.80	−4.425	<0.001
	B	541.20 ± 15.08	596.40 ± 10.75	4.432	<0.001
HRQoL	A	72.24 ± 1.62	79.20 ± 1.65	−4.415	<0.001
	B	72.16 ± 1.40	78.84 ± 1.54	4.412	<0.001
HbA1c (%)	A	7.34 ± 0.14	6.70 ± 0.09	−4.397	<0.001
	B	7.404 ± 0.124	6.74 ± 0.076	4.396	<0.001

Comparisons Between Two Intervention Approaches

There was no indication of significant difference when Mann-Whitney U test was performed in baseline scores for outcome measures between groups, confirming that both were representative of a similar population. After 12 weeks of treatment, post-treatment between group analysis indicated that all but one outcome was statistically similar between groups (i.e. FVC, FEV1, PEFR, 6MWT, HRQoL and HbA1c; all p-values >0.01) indicating both groups produced statistically similar improvements across the

outcomes assessed. However, the FEV1/FVC ratio demonstrated a statistically significant difference between groups (U=202.5, p=0.032), with Group A demonstrating a more favorable improvement than Group B in their ability to perform pulmonary gas exchange.

Table 3: Between Group Analysis Mann-Whitney U Test (Post-Intervention)

Variable	Group A (PCT) Post Mean ± SD	Group B (CST) Post Mean ± SD	U-value	p-value
FVC (L)	3.09 ± 0.184	3.13 ± 0.20	274.5	0.442
FEV1 (L)	2.59 ± 0.157	2.62 ± 0.14	277.5	0.490
FEV1/FVC (%)	83.52 ± 3.42	81.52 ± 2.85	202.5	0.032*
PEFR (L/s)	402.8 ± 21.5	406.8 ± 20.35	262.5	0.316
6MWT (m)	593.20 ± 11.80	596.4 ± 10.75	262.5	0.316
HRQoL	79.20 ± 1.65	78.84 ± 1.54	262.5	0.316
HbA1c (%)	6.70 ± 0.09	6.74 ± 0.076	253.5	0.213

** Both periodized circuit training (PCT) and conventional strength training (CST) had statistically significant effects*

DISCUSSION

A randomized, controlled trial compared the effectiveness of 12 weeks of either periodized circuit training (PCT) or conventional strength training (CST) on not only pulmonary function but also cardiovascular fitness, glycemic control, and health-related quality of life for people with T2DM. As with all outcomes, both groups exhibited significant improvements within themselves as a group; however, the only significant difference (favoring PCT) between the two groups was with regard to the FEV1/FVC ratio.

For pulmonary function, both groups showed statistically significant improvements in FEV1, FEV1/FVC ratio, and PEFR; however, FVC did not show a statistically significant difference between groups A and B. The FEV1/FVC ratio improvements achieved by Group A (<0.032) as compared to Group B are consistent with another study that found positive effects from combined aerobic/ resistance training (A/R) on improving spirometric parameters for T2DM through increased respiratory muscle endurance and decreased systemic inflammation21. Walking and jogging during the aerobic portion of PCT likely assisted in the bronchodilation process and helped promote cardiorespiratory coupling, while increasing the strength of the diaphragm, therefore improving the efficiency of the airway beyond what was achieved by doing the CST alone[22]. These findings are contradicted by an alternate study that reported no difference in airway changes between A/R and R training and suggest that variations in the intervention methods or subjects, in combination with variations present within each of the samples used in those studies, may account for the differing results characteristics[23].

Both groups had equivalent, statistically significant improvements in distance (6MWT) regarding cardiovascular fitness with no statistically significant difference in distance covered between groups. This finding is consistent with other research showing resistance training results in improved functional walking capacity due to increased muscle-related energy production24. In both groups of participants, the aerobic elements of PCT replicated the demands of the 6-MWT protocol, while the resistance exercises produced strengthened/lowered limb muscle fatigue resulting in similar functional benefits over the short term. In addition to cardiovascular fitness, both groups experienced statistically significant reductions in HbA1c with no statistically significant group differences. This finding is also consistent with the body of literature on both resistance training and combined training as methods for increasing insulin sensitivity via GLUT4 translocation and AMPK activation25. Rhythmic large muscle aerobic exercises performed as part of the PCT protocol provided for acute, non-insulin-dependent glucose clearance while the resistance components in both groups allowed for similar muscle contractions resulting in increased post-exercise glucose uptake despite training at similar frequencies[26]. Similar improvements occurred in human quality of life measures for both groups with no statistically significant differences between groups. The improvement in quality of life as assessed by human quality of life measures is consistent with literature of evidence that exercise has a globally positive effect on human quality of life through improved physical and mental functioning[27-28]. Conclusion regarding the sustainability of the improvements observed is limited due to the home-based and balanced approach to the survey follow-up design.

The main strengths of this study are that it is a randomized controlled study that uses validated outcome measures in multiple physiological domains and home-based delivery model which increases its real-world applicability. But the results are to be interpreted with caution due to the short follow up of 12 weeks, lack of adherence monitoring and small size of the sample, and the lack of control over dietary and pharmacological confounders. Subsequent studies with longer intervention durations, larger multi-center samples with objective compliance monitoring, and include biomarkers, such as CRP and VO_2 peak, should be used in future studies to further explain the underlying physiological mechanisms of PCT in T2DM populations.

CONCLUSION

The study supports that both periodized circuit training and conventional strength training are effective interventions for improving pulmonary function, cardiovascular fitness, control of blood sugar levels and health-related quality of life in people with T2DM. However, periodized circuit training demonstrated a significantly superior improvement in airway efficiency, as reflected by the FEV1/FVC ratio, suggesting it may offer broader pulmonary benefits. Clinicians may consider PCT as a preferred home-based exercise modality for comprehensive T2DM management, though longer trials are warranted to confirm sustained outcomes.

Ethical Approval

The study was registered at ClinicalTrials.gov (Identifier: NCT07484672) and received ethical approval from Riphah International University (Protocol Record No. REC/RCR&AHS/24/0388). Data were collected from May 2025 to November 2025.

Author Contributions

HJ: Conception & Design, Data Collection, Manuscript Writing, and Critical Revision

HMG: Conception & Design, Data Analysis & Interpretation, and Final approval

SM: Data Collection and Final approval

MU: Data Analysis and Final approval

SKA: Conception & Design, Data Collection, and Critical Revision

Conflict of Interests

No conflict of interest.

Data Availability

Data will be available upon request.

Funding Source

No sources

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Prevalence of Computer Vision Syndrome and Its Associated Risk Factors Among Computer Science Students at The University of Sargodha, Pakistan: A Cross-Sectional Study

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ABSTRACT

Background: Computer Vision Syndrome (CVS) or Digital Eye Strain is a widely-known ailment that is the result of excessive use of digital displays and causes ocular and musculoskeletal discomfort. Technology based programs, especially to university students are highly vulnerable. The purpose of the study was to identify the prevalence and the risk factors of CVS among computer science students in the University of Sargodha.

Methods: The study was descriptive and cross-sectional with a study period of four months among 177 students who were selected using the convenience sampling technique. The self-administered questionnaire was a structured questionnaire that was self-administered and it included questions that evaluated the following: demographics, screen usage patterns, ergonomic practices and CVS symptoms. The severity of symptoms was measured as mild, moderate or severe symptoms.

Results: The prevalence of CVS was 71.2% (n = 126). The mean age was 21.51 ± 1.92 years, with 57.6% males. The most prevalent symptoms were dry eyes (35.6%), headache (31.6%), and neck and shoulder pain (25.4%), with severe symptoms being most frequently reported on neck and shoulder pain (22.0%). Almost half of the participants (49.7) were using a 20-20-20 rule (66.7). Significant relationships were found between daily screen time and CVS severity ($\chi 2 = 325.843, 15, 0.001$).

Conclusion: CVS is very common among the students. Such modifiable factors like long screen time, poor ergonomics and low awareness play a significant role. Educating on eye health, ergonomics training and regular screening should be incorporated.

Keywords: *Computers, Eyestrain, Ocular, Vision*

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INTRODUCTION

Computer Vision Syndrome (CVS) or Digital Eye Strain (DES) can be defined as the complex of eye and vision symptoms in relation to near-vision tasks that are experienced during or in relation to computer use and the use of other digital devices, including desktop computers, laptops, tablets, and smartphones[1]. The resulting screen time has placed a student population at a higher risk to CVS in recent years, especially during and after the COVID-19 pandemic, when academic instruction shifted to online platforms[2-3].

Among computer users, CVS is estimated to affect about 60 million people, with nearly one million new cases reported each year[4]. It has been noted that its prevalence among computer users ranges between 63% and 89%, establishing it as one of the leading occupational health hazards of the twenty-first century[5]. The prevalence rates among university students have been found to be particularly high: 94% of medical students in Saudi Arabia have reported at least one of the CVS symptoms[6], and 77% of students surveyed in China have reported similar complaints7. Among Thai university students who study online during COVID-19, self-reported CVS has been found to be significantly high[3]. The

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most common ocular symptoms are dry eyes, eye strain, burning, redness, blurred vision, and diplopia, whereas the most common systemic complaints include headache, neck and shoulder pain, and musculoskeletal discomfort.[8,9] Headache and dry eyes have always been the most common reported symptoms with diverse study populations[6,10]. Also, CVS has been demonstrated to lower work and academic productivity rates by up to 40%, indicating its substantial socioeconomic cost[11]. A number of risk factors have been determined to be related to the development of CVS. They include a long period of computer use, low ambient light, incorrect viewing distance and angle, uncorrected refractive errors, reduced blink rate, and improper workstation ergonomics[5,12] .Gender of females, lack of break time and pre-existing ocular conditions have also been linked to increased predisposition to CVS.[10,13] Physiologically, the decrease in blink rate between a normal 16-18 blinks/minute to an approximate of 6-8 blinks/minute in prolonged screen time results in incomplete tear film replacement, corneal drying and resultant ocular discomfort[14]. With prolonged accommodative effort, ciliary muscle fatigue also increases, thereby leading to asthenopia and blurred vision. Although there is significant literature on CVS in Western and East Asian student populations, the literature on South Asian academic settings is limited. In Pakistan and within the specific population of Pakistani university students, there has been an increased dependence on digital devices as a means of academic learning and research, as well as for socializing, but the burden of CVS in this population has not been sufficiently quantified[15]. The University of Sargodha is a large state-run institution in Punjab, which harbors thousands of students of all kinds, with various degrees of screen exposure, which makes it a perfect place to conduct such an investigation. The local prevalence and related risk factors of CVS should be understood to help develop context-specific preventive methods and evidence-based guidelines to screen use among students. Hence, the current study was carried out, so as to estimate the prevalence rate and the risk factors of Computer Vision Syndrome among the University of Sargodha students.

METHODOLOGY

Design and Setting

It was a descriptive cross sectional observational study which was conducted on computer science students at university of Sargodha, Punjab, Pakistan. The university was chosen as the study site because of the large number of students enrolled in the university at various disciplines having significant and diverse computer use. The study was conducted for four months and the ethical approval was obtained from the University of Sargodha under Ethics Code: 3152024.

Sample Size

The sample size was determined according to the standard formula for proportion-based cross-sectional studies:

$$n = z^2 \times P(1 - P) / d^2$$

Based on a similar study done among computer science students of an engineering college in Bengaluru, India with a reference prevalence of 86.67%, the calculated sample size for a 95% confidence level (z = 1.96) and margin of error (d = 0.05) was a total of 177 students were thus involved in the study.

Sampling Technique

The non-probability sampling method of convenience sampling technique was used to recruit those participants who were eligible for the study from the Department of Computer Science, University of Sargodha. Students who were on site during data collection and met the selection criteria were approached for enrollment.

Inclusion Criteria

The age of the students was between 18 to 30 years and they were the students of computer science program in University of Sargodha. The participants had to use a computer or digital screen at least five hours a day and to have used a computer or digital screen on a regular basis for four months prior to data collection[16].

Exclusion Criteria

Students were excluded if they had known ocular disease, history of cervical head injury or history of neck, back or shoulder injury. In addition, those students who refused to give informed consent and those who did not volunteer to participate were excluded from the study[16].

Data Collection Tool

The questionnaire was divided into two parts and based on previously validated questionnaires in

similar CVS studies[17]. The first part included demographic and screen use data including age, gender, hours per day of screen use, frequency of computer use, average break length, distance between eyes and screen, sitting position, ambient light level in the room, screen brightness, and knowledge of the 20-20-20 rule. Other data recorded included corrective eyewear (spectacles, contact lenses), topical eye drops (dry eye) and history of ocular disease or head injury.

The second section evaluated the four cardinal CVS symptoms of headache, dry eyes, blurred vision and neck and shoulder pain. The severity of the symptoms was rated as follows: Mild (transient, lasting only a few minutes); Moderate (lasting a few hours, but subsiding on rest or sleep); and Severe (persistent, requiring medical attention).

Data Collection Procedure

Ethical approval was obtained from the Institutional Review Board (IRB) prior to commencement of the study. Written informed consent was obtained from each participant after the study purpose, procedures, and confidentiality measures had been clearly explained. Participation was entirely voluntary, and participants were permitted to withdraw at any point without consequence. Questionnaires were distributed in-person during class hours and collected on the same day to ensure a high response rate. Anonymity of responses was maintained throughout the data collection process.

Statistical Analysis

All data were entered and analyzed using IBM SPSS Statistics, Version 22.0 for Windows (IBM Corp., Armonk, NY, USA). Categorical variables, including demographic characteristics, screen-use habits, and CVS symptom frequencies, were summarized using frequencies and percentages. The association between CVS symptoms and potential risk factors was evaluated using the Chi-square test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Demographic Description

A total of 177 computer science students at the University of Sargodha were enrolled in this cross-sectional study. Data were collected via a self-administered questionnaire and analyzed using SPSS version 22. The mean age of participants was 21.51 ± 1.92 years. The majority were male (n = 102, 57.6%), with 75 females (42.4%). Demographic characteristics, device usage patterns, and clinical history are summarized in Table 1.

Table 1. Demographic Characteristics, Device Usage, and Clinical History of Participants (n = 177)

Variable	Category	n (%)
Gender	Male	102 (57.6%)
	Female	75 (42.4%)
Age (years) Mean ± SD: 21.51 ± 1.92	18–20	64 (36.2%)
	21–22	54 (30.5%)
	23–25	59 (33.3%)
Device Used Most Frequently	Laptop	83 (46.9%)
	Android Mobile	68 (38.4%)
	Tablet/iPad	22 (12.4%)
	Desktop PC	4 (2.3%)
Daily Screen Time	1–2 hours	11 (6.2%)
	3–4 hours	44 (24.9%)
	4–5 hours	73 (41.2%)
	>6 hours	49 (27.7%)
Pre-existing Ocular Disease	Yes	51 (28.8%)
	No	126 (71.2%)
Use of Glasses/Contact Lenses	Yes	85 (48.0%)
	No	92 (52.0%)
Use of Topical Eye Drops	Yes	67 (37.9%)
	No	110 (62.1%)

SD = Standard deviation.

Screen Use Habits and Ergonomic Risk Factors

Regarding device usage, the laptop was the most frequently used screen (46.9%), followed by android mobile (38.4%). The majority of students (41.2%) spent 4–5 hours daily on screens, while 27.7% spent more than 6 hours. Nearly half of participants (49.7%) reported a bent-back posture during screen use, and only 24.9% maintained an upright posture. Viewing distance was suboptimal in 24.9% of students who sat closer than 45 cm, while 27.1% were unaware of their screen distance. Most participants (52.0%) worked in a bright room, while 40.1% used a bright screen setting. Even though 72.3% said that they took breaks, only 11.3% did it every 15 minutes as they should have done. More importantly, two-thirds of students (66.7%) were not aware of the 20-20-20 rule. Table 2 outlines these ergonomic risks factors.

Table 2. Ergonomic and Screen Use Risk Factors Among Study Participants (n = 177)

Risk Factor	Category	n (%)
Sitting Posture	Upright / Straight back	44 (24.9%)
	Bent back	88 (49.7%)
	Lying down	45 (25.4%)
Eye-to-Screen Distance	<45 cm	44 (24.9%)
	40–76 cm (optimal)	69 (39.0%)
	>76 cm	16 (9.0%)
	Don't know	48 (27.1%)
Screen Brightness	Very bright	22 (12.4%)
	Bright	71 (40.1%)
	Dull	69 (39.0%)
	Very dull	15 (8.5%)
Room Illumination	Very bright	15 (8.5%)
	Bright	92 (52.0%)
	Dull	54 (30.5%)
	Dark	16 (9.0%)
Takes Break During Screen Use	Yes	128 (72.3%)
	No	49 (27.7%)
Break Frequency	Every 15 min	20 (11.3%)
	Every 30 min	79 (44.6%)
	After 1 hour	27 (15.3%)
	No break	51 (28.8%)
Awareness of 20-20-20 Rule	Aware	59 (33.3%)
	Not aware	118 (66.7%)

20-20-20 rule: every 20 minutes, look at something 20 feet away for 20 seconds

CVS Symptom Profile and Severity

Table 3 shows the severity of each CVS symptom in each of the four grades (no symptom, mild, moderate, severe). The most clinically severe symptom was a neck and shoulder pain, with 22.0% of the participants reporting severe grades. The severity of headache was mostly mild (40.7%), and moderate (38.4%). The moderate severity reported by 34.5% of students on dry eyes is moderate. The most prevalent mild symptom was burning of the eyes (49.7%), and the highest proportion of participants who were asymptomatic had eye redness (38.4%).

Table 3. Severity Distribution of Computer Vision Syndrome Symptoms among Study Participants (n = 177)

Symptom	No Symptom n (%)	Mild n (%)	Moderate n (%)	Severe n (%)
Headache	32 (18.1%)	72 (40.7%)	68 (38.4%)	5 (2.8%)
Burning Eye Sensation	48 (27.1%)	88 (49.7%)	36 (20.3%)	5 (2.8%)
Eye Redness	68 (38.4%)	68 (38.4%)	40 (22.6%)	1 (0.6%)
Blurred Vision	51 (28.8%)	73 (41.2%)	50 (28.2%)	3 (1.7%)
Dry Eyes	44 (24.9%)	70 (39.5%)	61 (34.5%)	2 (1.1%)
Neck & Shoulder Pain	17 (9.6%)	48 (27.1%)	73 (41.2%)	39 (22.0%)

Severity grading: Mild = transient, lasting minutes; Moderate = lasting hours, resolves with rest; Severe = requires medical attention.

Prevalence of CVS and Association with Screen Time

The overall prevalence of CVS in this study was 71.2% (n = 126). The only most frequently reported dominant symptom (35.6%), was the dry eyes, and in turn, headache (31.6%), neck and shoulder pain (25.4%). The relationship between daily hours of screen time and the prevalent symptoms experienced in CVS were statistically significant (Pearson χ 2 = 325.843, df = 15, $p < 0.001$), which implies that an increase in the hours spent on screens was strongly correlated with an increase in the number of symptoms experienced in CVS. All these findings are summarized in Table 4.

Table 4. Prevalence of CVS, Most Common Symptoms, and Association with Daily Screen Time

VARIABLE	Category / Finding	n (%)	χ^2 / p-value
Prevalence of CVS	CVS Present	126 (71.2%)	–
	No CVS	51 (28.8%)	–
Most Common Symptom	Dry Eyes	63 (35.6%)	–
	Headache	56 (31.6%)	–
	Neck & Shoulder Pain	45 (25.4%)	–
	Burning Eye Sensation	8 (4.5%)	–
	Blurred Vision	4 (2.3%)	–
	Eye Redness	1 (0.6%)	–
Association: Daily Screen Time × CVS Symptoms	Pearson Chi-Square	325.843 (df=15)	p < 0.001

CVS = Computer Vision Syndrome; χ^2 = Pearson Chi-Square; df = degrees of freedom. $p < 0.05$ considered statistically significant.

DISCUSSION

The present study established a high prevalence (71.2%) of digital eye strain among computer science students in the University of Sargodha, which confirmed a high level of digital eye strain

in this academic group. This statistic is generally standard with the previous estimates observed in university students across the world, which are between 63% and 89% depending on the population being studied and the diagnostic criteria used[5]. Our study revealed a prevalence similar to that of 71.6% reported in the medical students of Kathmandu, Nepal[18], and comparable to that of Saudi Arabian undergraduate students where 94% reported at least one CVS symptom[2]. It is, though, less than the 86.67% prevalence of the same, recorded amongst engineering students in Bengaluru, India[12], that was used as the reference in calculating the sample size in the present study. This peripheral difference can capture a difference in the daily patterns of exposure to the digital device, the intensity of academic workload, and the type of digital devices that is used in the majority of cases between the two groups.

In our study, male students constituted the majority (57.6%), with a mean age of 21.51 ± 1.92 years. No statistically significant association was identified between gender and CVS occurrence, which is consistent with findings reported in a Karachi-based study among undergraduate medical students[15]. However, this contrasts with some studies that have reported a higher susceptibility among females, possibly attributable to hormonal influences on tear film stability and greater symptom reporting tendencies[5]. The young age of our study participants is noteworthy, as early-onset CVS in this cohort may carry significant long-term implications for academic performance and ocular health if preventive measures are not adopted.

Dry eyes emerged as the most prevalent dominant symptom in this study (35.6%), followed by headache (31.6%) and neck and shoulder pain (25.4%). The predominance of dry eye symptoms is physiologically explained by the well-documented reduction in blink rate during sustained screen use — declining from a normal 16–18 blinks per minute to approximately 6–8 blinks per minute — which leads to incomplete tear film replenishment, corneal surface desiccation, and resultant ocular discomfort[14]. These findings are consistent with those reported among Sri Lankan office workers, where dry eyes (31.1%) and headache (45.7%) were the leading complaints[5], and with a study among Thai university students during COVID-19 online learning, which similarly identified dry eyes and headache as the most frequently reported symptoms[3]. The moderate-to-severe grading of dry eyes observed in 35.6% of our participants underscores the clinical significance of this symptom and the need for targeted intervention.

Headache was the second most dominant CVS symptom (31.6%), reported as mild by 40.7% and moderate by 38.4% of participants. This trend is in line with results of other related studies. The cause of headache related to prolonged computer use is the repeated accommodative and vergence demands being placed on the visual system, where constant refocusing between the screen and other near objects places stress on the ciliary muscles and extraocular musculature which eventually results in frontal or periorbital headache[9]. A survey conducted among medical students in Riyadh also found headache as the most reported CVS complaint[2]. The overall implications of these findings are that more structured eye rest periods should be implemented in schools.

The most severe symptom in the current study was neck and shoulder pain with 22.0% of students reporting severe grades which is a burden of severe symptoms than the current study found any other CVS symptom. This observation is comparable to reports in several studies that reported musculoskeletal complaints as a major non-ocular aspect of CVS.[10,19] Sitting posture was very popular in our cohort with 49.7% of the students assuming a bent back position when they were using the screen and only 24.9% maintaining an upright posture. The sustained forward-leaning, and bent postures during a prolonged screen use are well known causes of cervical strain, excessive load on upper trapezius muscles, and musculoskeletal pain[20]. These ergonomic shortcomings in a young learner population should be subjected to corrective interventions.

A statistically significant association was identified between daily screen time and CVS symptom burden ($\chi^2 = 325.843$, df = 15, $p < 0.001$), with greater screen exposure correlating with increased symptom frequency and severity. This is in line with the wider literature: Al Tawil and colleagues have found that students who spend over four hours a day at screens are much more likely to develop CVS,[10] and dose-response relations between screen exposure and CVS symptoms have been reported in Ethiopian and Indian student populations[4,21]. In our research,

41.2 % of students spent 4-5 hours per day on screens, and 27.7 % of students spent more than 6 hours per day on screens, a trend that places the majority of the sample in the high-risk exposure category.

A number of adjustable ergonomic risk factors were common in this study population. It was found that sub-optimal viewing distances existed in 24.9% of participants who sat within 45 cm of their screen and in 27.1% of participants who were completely unaware of what they were watching. This is because 52.5% of students rated the brightness of the screen as very bright or bright. These environmental factors have been repeatedly discovered to play a role in CVS among similar populations[12,22]. Importantly, 66.7% of students were unaware of the 20-20-20 rule which is one of the widely recommended preventive measures which advises one to look at an object that is 20 feet away 20 seconds every 20 minutes of screen use. This observation is similar to the low levels of preventive knowledge of a study published by the Sohag University[23], and outlines a great deficiency in health education in the university context which needs to be filled by systematic creation of awareness[24-25].

Although nearly half of the participants (48.0%) were wearing corrective eyewear (spectacles or contact lenses), the prevalence of CVS still remained high at 71.2% indicating that the symptom load in this population may not be fully attributed to refractive errors that are uncorrected or incorrectly corrected. This aligns with other studies it was discovered that that university students realized high levels of CVS despite relatively high levels of visual correction[12]. About 37.9% of students were self-administering topical eye drops to dry eyes, which is a significant proportion of students already experiencing symptomatic ocular discomfort severe enough to justify self-treatment, but without being formally clinically assessed. This further highlights the significance of regular optometric screening in university health care. This research has a number of limitations which must be noted. The CVS symptoms were completely self-reported through a structured questionnaire and no objective clinical ocular examination was conducted. The actual screen-use behavior of the participants, such as the accuracy of viewing distance and posture was not directly observed.

The research was confined to a single department at one university with the use of convenience sampling, which limits the application and generalization of the results to the general student population in Pakistan. In addition, the cross-sectional study design does not allow any causal relationship between the risk factors identified and the development of CVS. It is suggested to enhance the evidence base by future studies that will utilize larger multi-institutional samples and objective clinical measures and longitudinal follow-up.

CONCLUSION

The prevalence of Computer Vision Syndrome is very high among the computer science students of the University of Sargodha with a prevalence of about three quarters of the study population (71.2%). The most burdensome symptoms were dry eyes, headache, and pain in the neck and shoulders, although all of them proved to be the same in severity. Long-term daily screen time was considerably linked with CVS symptom burden. Bad ergonomic habits such as bent-back posture, less than optimum viewing distances and near universal ignorance of the 20-20-20 rule are critical and modifiable risk factors in the population. These results suggest the need to incorporate structured ergonomic education, periodic optometric screening and evidence-based screen-use policies in university health and academic policies. It is advisable to conduct larger multi-center studies so that the generalizability of these findings to the population of students in Pakistan can be made.

Ethical Approval

The research protocol was approved by the ethical review committee of University of Sargodha under Ethics Code: 3152024

Author Contributions

MA: Conception & Design, Data Collection, and Critical Revision
MS: Conception & Design, Data Analysis & Interpretation, Manuscript Writing, and Final approval
MM: Conception & Design, Data Analysis & Interpretation, Manuscript Writing, Critical Revision and Final approval
SFZR: Data Collection, Data Analysis & Interpretation, and Manuscript Writing.
AA: Conception & Design, Critical Revision and Final approval

Conflict of Interests
No conflict of interest.

Data Availability
Data will be available upon request.

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Original Article

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Effect of Supervised Exercise Training on Oxidative Stress Markers and Inflammatory Profile in Patients with Chronic Obstructive Pulmonary Disease: A Randomized Controlled Trial

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ABSTRACT

Background: Chronic obstructive pulmonary disease (COPD) is a chronic respiratory condition characterized by progressive lung dysfunction, chronic oxidative stress and systemic inflammation, which impair exercise capacity and quality of life. This study compared the effect of a 12-week supervised combined aerobic and resistance training program with a 12-week home-based aerobic training on pulmonary function, oxidative stress and inflammatory markers in COPD patients.

Methods: This was a single blind, two arm parallel randomized controlled trial conducted at the Department of Physical Therapy, Dow University of Health Sciences, Ojha Campus, Karachi from February to October 2025. 106 COPD patients who were diagnosed by a pulmonologist were randomly divided into two groups of 53. Group A did supervise combined aerobic and resistance training 5 times a week for 12 weeks, while Group B did aerobic exercises at home, at the same frequency. Pulmonary function tests (VC, TLC, FEV1/VC), oxidative stress indicators (SOD, MDA), and inflammatory cytokines (IL-6, TNF-α) were used as outcome measures. Paired and independent t-tests were used to analyze data, and p values < 0.05 were considered statistically significant.

Results: Within-group improvements were significant for both groups for all variables measured ($p < 0.05$). But Group A showed a significantly better increase in VC, FEV1/VC ratio, SOD, MDA reduction and decrease in IL-6 and TNF-α compared to Group B ($p < 0.05$).

Conclusion: Group A shows greater improvement in pulmonary function, oxidative balance and inflammation than home-based AT, and should be considered as a preferred method for pulmonary rehabilitation in COPD patients.

Keywords: *COPD, Inflammatory Markers, Pulmonary Function Test, Vital Capacity*

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a huge health problem in the 21st century and is one of the most important causes of morbidity and mortality in the world. It affects approximately 10% of adults aged 30–79 years and is currently ranked as the third leading cause of death globally[1]. There are a number of significant systemic changes associated with COPD and the burden of COPD is also regionally variable, largely due to smoking prevalence, environmental pollution and access to health care[2]. Effective non-pharmacological management strategies are needed to address COPD beyond the respiratory changes.

Chronic oxidative stress and systemic inflammation are one of the hallmarks of COPD pathogenesis. Superoxide dismutase (SOD) is an important antioxidant enzyme and malondialdehyde (MDA) is a marker of lipid peroxidation and oxidative damage from a biochemical point of view[3]. Watson et al.

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observed that few studies with suboptimal data were available in a systematic review on the effect of pulmonary rehab on redox status, and they believed this was due to the lack of well-designed studies[4]. In COPD patients' systemic inflammation, driven by cytokines like interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α), also plays a role in muscle dysfunction, exercise capacity and pulmonary function[5]. Pulmonary rehabilitation (PR) is an important part of COPD treatment. Aerobic exercise increases antioxidant defenses and decreases inflammation, oxidative stress and apoptosis, and resistance training increases antioxidant defenses and mitochondrial function[6-8], combined training has been shown to have synergistic effects such as decreased protein carbonyl and improved functional outcomes[9].

There is a lack of research on the role of supervision in the delivery of exercise. There was greater improvement in exercise capacity with supervised rehabilitation programs than with home-based programs[10], but the effect of these programs on oxidative stress and inflammatory markers remains uncertain, and lung function parameters such as VC, TLC, and FEV1/VC have not been extensively studied. A structured progression and monitoring of rehabilitation may be suboptimal at home and can be achieved through supervised rehabilitation[11].Furthermore, supervised resistance training has been associated with a decrease in inflammatory markers and improvement in pulmonary function in elderly people with COPD[12]. Thus, a randomized controlled trial was performed to compare the effect of 12 weeks of supervised combined aerobic and resistance training, and home-based aerobic training on oxidative stress markers (SOD, MDA), inflammatory cytokines (IL-6, TNF-α) and pulmonary function indices (VC, TLC, FEV1/VC).

METHODOLOGY

Study Design and Setting

This single-blind, two-arm parallel randomized controlled trial (RCT) was conducted at the DUHS F&SRC, Dow University of Health Sciences, Ojha Campus. The study was carried out over a period of nine months, from January 2025 to December 2025.

Sample Size

A total sample of n=106 participants was enrolled in this study, calculated using G*Power software (version 3.1) at a significance level of α = 0.05, statistical power of 80%, and based on the value of FEV1/ FVC of second research period 67.43±15.31 for study group and 58.023±19.12 for control group[9]. Participants were randomly allocated into two equal groups of 53 each: Group A (Supervised Combined Training) and Group B (Home-Based Aerobic Training) using a computer-generated randomization sequence with sequentially numbered sealed opaque envelopes to ensure allocation concealment.

Selection Criteria

Inclusion Criteria: Participants were included if they (i) had a confirmed diagnosis of COPD established by a registered pulmonologist based on spirometric criteria (post-bronchodilator FEV1/FVC < 0.70) in accordance with GOLD guidelines; (ii) were in clinical remission with no acute exacerbation in the preceding four weeks; (iii) were aged between 40 and 60 years; (iv) were medically stable and cleared by their treating pulmonologist to participate in an exercise program; and (v) were able to provide written informed consent and commit to the 12-week intervention period.

Exclusion Criteria: Participants were excluded if they (i) had a concurrent diagnosis of uncontrolled cardiovascular disease, musculoskeletal disorder, or neurological condition that would contraindicate exercise participation; (ii) had a history of exacerbation requiring hospitalization within the previous four weeks; (iii) were currently enrolled in any other formal pulmonary rehabilitation program; (iv) had any significant comorbidity that would independently affect oxidative stress or inflammatory markers, such as active malignancy or autoimmune disease; (v) were using systemic corticosteroids or immunosuppressive therapy at the time of enrollment; or (vi) were unable to perform spirometry reliably.

Data Collection Procedure

Baseline assessment was carried out in less than one week before the start of the intervention. A trained assessor who was blinded to group assignment recorded all outcome measures at the point of baseline (Week 0) and post-intervention (Week 12).

Pulmonary Function Testing (PFT):

Spirometric testing was done using a calibrated spirometer in accordance with American Thoracic Society/European Respiratory Society (ATS/ERS) standardization guidelines. Three measures were documented: Vital Capacity (VC), Total Lung Capacity (TLC) and FEV1/VC ratio. The best of three acceptable maneuvers was recorded for each participant.

Oxidative Stress Markers:

Each of the participants was placed under fasting conditions and the venous blood sample (5 mL) was collected. The amount of SOD activity in serum samples was determined using a colorimetric assay kit of enzyme activity, and the level of Malondialdehyde (MDA) was measured through the thiobarbituric acid reactive substances (TBARS) technique. The samples were centrifuged, aliquoted and stored at -80 o C until batch analysis.

Inflammatory Markers:

The serum levels of Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-α) were measured using commercially made enzyme-linked immunosorbent assay (ELISA) kits in accordance with the manufacturer instructions.

Intervention Protocol

Group A: Supervised Combined Aerobic and Resistance Training:

Group A participants had a structured 12 weeks supervised combined aerobic and resistance exercise program which was conducted at the physical therapy outpatient department, OJHA Campus, 5 sessions a week. The program consisted of three aerobic sessions and two sessions on resistance training per week.

The aerobic sessions were done on a motorized treadmill or stationary cycle ergometer, with the intensity of the training being prescribed as 70% of the Maximum Heart Rate (MHR) and calculated using the formula: MHR = 220- age. Each session, the pulse oximeter was constantly used to monitor heart rate. All aerobic exercises took 30-40 minutes with warm-up (5 minutes low intensity) and cool-down (5 minutes low intensity).

The resistance training was performed on two nonconsecutive days in a week. The individualization of the training load was done using the One-Repetition Maximum (1RM) method, which was measured at baseline in each relevant exercise. The participants were trained at 60-70 % of their own 1RM with progressive overload every two weeks. The first day of resistance was focused on upper limbs and back, including the exercises of seated dumbbell shoulder press, seated cable row, and bicep curls. Day 2 Resistance was dedicated to low limbs; leg press, knee extension, and seated leg curl were all incorporated. The resistance sessions were divided into 2-3 sets of 10-12 repetitions of exercises separated by a one-to-two-minute relaxation period. The sessions were directly supervised by a qualified physical therapist who observed technique, progression of load, and tolerance in the participants during the intervention.

Group B: Home-Based Aerobic Training:

Home-Based Aerobic Training: Group B participants completed 12 weeks of aerobic training at home, five sessions per week, and at a prescribed intensity of 70% of their MHR. The participants were trained on how to use a personal pulse oximeter to self-monitor the heart rate. Modality of exercise entailed walking or stationary cycling of 30-40 minutes per session. Participants were given a standardized written exercise guide and a weekly supervisory phone call with the research physical therapist to monitor compliance, address concerns and reinforce compliance. No formal resistance training was included in this group.

Statistical Analysis

The entered and analyzed data were SPSS version 26 (IBM Corp., USA). Shapiro-Wilk test was used to determine the normality of data. A frequency and percentage were used for descriptive analyses. Within group analyses paired t test was run and for between group comparison independent t-test was performed. Values were significant at <0.05.

Ethical Consideration

The study was approved by the Ethical Review Committee of the Foundation of Medical Research and Laboratories (IRB Protocol No.: FMRL-IRB/2025/033) before the beginning of the study. All participants gave informed written consent prior to enrolment. The trial was carried out in compliance with the

principles of the Declaration of Helsinki.

RESULTS

A total of 106 participants with confirmed COPD were enrolled and randomized into two groups of 53 each. No significant dropouts were recorded during the 12-week intervention period. The two groups were comparable at baseline with respect to all demographic and clinical variables, as shown in Table 1.

The mean age of participants in Group A was 52.4 ± 5.8 years and in Group B was 51.8 ± 6.2 years. Males constituted the majority in both groups (66% and 62% respectively). No statistically significant between-group differences were observed for age, sex, BMI, smoking status, or GOLD staging (all $p > 0.05$), confirming successful randomization.

Table 1. Baseline Demographic and Clinical Characteristics of Participants

Variable	Group A (n=53)	Group B (n=53)	p-value
Age (years)	52.4 ± 5.8	51.8 ± 6.2	0.611
Gender	35 (66%)	33 (62%)	0.720
BMI (kg/m²)	24.6 ± 3.1	25.1 ± 3.4	0.432
Smoking Status	38 (71.7%)	36 (67.9%)	0.721
GOLD Stage II	28 (52.8%)	27 (50.9%)	0.845
GOLD Stage III	25 (47.2%)	26 (49.1%)	0.845
Disease Duration (years)	6.2 ± 2.4	6.5 ± 2.7	0.558

Group A = Supervised Combined Aerobic a Resistance Training; Group B = Home-Based Aerobic Training; BMI = Body Mass Index; GOLD = Global Initiative for Chronic Obstructive Lung Disease. Mean±; n (%)

Pulmonary function test parameters at baseline and post-intervention for both groups are presented in Table 2. Within-group analysis revealed statistically significant improvements in VC, TLC, and FEV1/VC in both groups following the 12-week intervention (all $p < 0.05$). However, between-group analysis demonstrated that Group A achieved significantly greater.

Table 2. Within and Between Group Comparison of Pulmonary Function Test Parameters

Parameter	Group A		Within-Group p	Group B		Within-Group p	Between-Group p
	Pre	Post		Pre	Post		
VC (L)	2.81 ± 0.42	3.24 ± 0.38	<0.001	2.78 ± 0.45	2.97 ± 0.41	<0.001	0.002
TLC (L)	6.84 ± 0.71	6.52 ± 0.64	<0.001	6.79 ± 0.68	6.66 ± 0.71	0.023	0.018
FEV1/VC (%)	56.8 ± 8.2	63.4 ± 7.6	<0.001	57.1 ± 7.9	60.2 ± 8.1	<0.001	0.014

VC = Vital Capacity; TLC = Total Lung Capacity; FEV1/VC = Forced Expiratory Volume in one second to Vital Capacity ratio. Within-group comparison by paired samples t-test; between-group comparison by independent samples t-test. Significance set at $p < 0.05$, Mean ± SD

The results of oxidative stress markers are presented in Table 3. At baseline, both groups demonstrated comparable levels of SOD and MDA ($p > 0.05$). Following the 12-week intervention, Group A showed a significantly greater increase in SOD activity (0.88 ± 0.21 to 1.34 ± 0.28 U/mL; $p < 0.001$) and a significantly greater reduction in MDA levels (4.38 ± 0.82 to 2.74 ± 0.61 µmol/L; $p < 0.001$) compared to Group B, which showed more modest within-group changes (SOD: 0.91 ± 0.19 to 1.08 ± 0.22 U/mL; MDA: 4.42 ± 0.79 to 3.51 ± 0.73 µmol/L). Between-group differences for both SOD and MDA were highly significant ($p < 0.001$), indicating superior attenuation of oxidative burden in the supervised combined training group. Improvements across all three spirometric parameters compared to Group B (all $p < 0.05$). The FEV1/VC ratio improved from 56.8 ± 8.2% to 63.4 ± 7.6% in Group A, compared to an

Table 3. Within and Between Group Comparison of Oxidative Stress Markers

Parameter	Group A		Within-Group p	Group B		Within-Group p	Between-Group p
	Pre	Post		Pre	Post		
SOD (U/mL)	0.88 ± 0.21	1.34 ± 0.28	<0.001	0.91 ± 0.19	1.08 ± 0.22	<0.001	<0.001
MDA (µmol/L)	4.38 ± 0.82	2.74 ± 0.61	<0.001	4.42 ± 0.79	3.51 ± 0.73	<0.001	<0.001

SOD = Superoxide Dismutase; MDA = Malondialdehyde. Within-group comparison by paired samples t-test; between-group comparison by independent samples t-test. Significance set at $p < 0.05$, Mean ± SD

improvement from 57.1 ± 7.9% to 60.2 ± 8.1% in Group B, with the between-group difference reaching statistical significance (p = 0.014).

Inflammatory marker results are presented in Table 4. Both groups demonstrated significantly elevated baseline concentrations of IL-6 and TNF-α consistent with established systemic inflammation in COPD, with no significant between-group difference at baseline ($p > 0.05$). Following the intervention, statistically significant within-group reductions were observed in both IL-6 and TNF-α concentrations in both groups (all $p< 0.001$). Group A exhibited substantially greater reductions in IL-6 (16.42 ± 4.21 to 9.18 ± 3.14pg/mL) and TNF-α (22.36 ± 5.14 to 13.74 ± 4.28pg/mL) compared to Group B (IL-6: 16.87 ± 4.53 to 13.24 ± 3.87 pg/mL; TNF-α: 23.01 ± 5.62 to18.42 ± 4.91 pg/mL). Between-group comparisons for both inflammatory markers were statistically significant ($p < 0.001$), demonstrating the superior anti-inflammatory effect of the supervised combined training protocol.

Table 4. Within and Between Group Comparison of Inflammatory Markers

Parameter	Group A		Within-Group p	Group B		Within-Group p	Between-Group p
	Pre	Post		Pre	Post		
IL-6 (pg/mL)	16.42 ± 4.21	9.18 ± 3.14	<0.001	16.87 ± 4.53	13.24 ± 3.87	<0.001	<0.001
TNF-α (pg/mL)	22.36 ± 5.14	13.74 ± 4.28	<0.001	23.01 ± 5.62	18.42 ± 4.91	<0.001	<0.001

IL-6 = Interleukin-6; TNF-α = Tumour Necrosis Factor-alpha. Comparison within a group by paired samples t-test; between groups by independent samples t-test. Significance level of $p < 0.05$.

DISCUSSION

The current randomized controlled trial compared the effects of 12 weeks of supervised combined aerobic and resistance exercise versus home-based aerobic exercise alone on pulmonary function, oxidative stress markers, and systemic inflammatory cytokines in patients with COPD. The main conclusion of the given study is that both of the interventions resulted in statistically significant within-group improvements in all of the measured outcomes; nevertheless, the supervised combined training group was characterized by significantly better gains in VC, FEV1/VC, SOD, MDA, IL-6 and TNF-alpha in comparison with the home-based aerobic training group. All these findings contribute to the argument that the modality, structure, and supervision of the exercise delivery is a critical determinant of the therapeutic effect of the intervention in COPD management, and that combined training under professional supervision is a more effective intervention than is the aerobic exercise performed independently at home.

The high rates of improvement in pulmonary functioning in both groups are aligned with the expanding literature that exercises-based pulmonary rehabilitation is a modifier of ventilatory mechanics in COPD. Group A, as represented in the current work, showed a more significant increase in VC and an increase in the

FEV1/VC, in addition to a significant decrease in TLC - the latter to reflect a clinically significant attenuation of pulmonary hyperinflation, which is one of the cardinal pathophysiological processes of COPD. These results support earlier evidence that graded exercise rehabilitation of COPD patients results in significant improvements in lung volumes and airflow indices, attributing the effect to improved function of respiratory muscles and reduced dynamic hyperinflation[13].

The relatively lesser spirometric improvements recorded in Group B though statistically significant, imply that aerobic exercise performed at home without resistance training is not adequate to maximally rehabilitate respiratory mechanics. This is biologically plausible, where resistance training of upper limb and trunk musculature included in the protocol of Group A directly trains accessory respiratory musculature, thereby enhancing thoracic compliance and expiratory flow. It has further been demonstrated through lung transcriptomic analysis that aerobic training upregulates gene pathways governing pulmonary remodeling and inflammation resolution in COPD, providing a molecular basis for the spirometry improvements observed in both groups[6].

With respect to oxidative stress, Group A exhibited substantially greater increases in SOD activity and reductions in MDA concentrations relative to Group B. SOD, as the primary enzymatic antioxidant defense against superoxide radicals, is notably depleted in COPD, and its restoration following exercise reflects an upregulation of endogenous antioxidant capacity[3]. The significant increase in SOD activity in Group A is consistent with evidence that resistance training induces mitochondrial biogenesis and enhances the expression of antioxidant enzymes within skeletal muscle tissue[7]. MDA, as a terminal product of lipid peroxidation, is widely used as a surrogate marker of oxidative membrane damage, and its significant reduction in Group A supports the premise that combined modality training exerts a superior protective effect against ROS-mediated cellular injury compared to aerobic training alone[4]. It has similarly been reported that reductions in oxidative stress biomarkers, including lipid peroxidation indices, following structured exercise rehabilitation in patients with compromised pulmonary function are dose-dependent, with higher exercise volumes and multi-modal training producing greater biochemical responses[14]. The more modest but still significant reductions in MDA observed in Group B confirm that aerobic exercise independently contributes to redox modulation, as previously demonstrated in experimental COPD models[8]. However, the magnitude of benefit conferred by the addition of resistance training in Group A through its specific stimulation of skeletal muscle antioxidant enzyme systems appears to account for the statistically significant between-group difference observed in the present study.

The changes in the levels of IL-6 and TNF-a observed in both groups after the 12-week intervention are clinically significant, as the high levels of these cytokines are central to the maintenance of systemic inflammation, skeletal muscle catabolism and progression of the disease in COPD[5]. Group A showed significantly greater decreases in both of the markers, indicating that the combination of aerobic and resistance exercise has a synergistic anti-inflammatory effect that was greater than the effect of aerobic training alone. It has been documented that structured pulmonary rehabilitation in COPD patients produces significant reductions in circulating inflammatory markers over time, with the magnitude of the anti-inflammatory response correlating with the intensity and regularity of exercise sessions[15]. The proposed mechanisms underlying exercise-induced attenuation of systemic inflammation in COPD include the reduction of adipose tissue-derived cytokine production, modulation of skeletal muscle myokine signaling particularly through the release of anti-inflammatory IL-6 from contracting muscle fibers during acute exercise bouts and downregulation of NF-κB-mediated inflammatory pathways with chronic training adaptation[16]. The progressive resistance component of Group A's protocol likely contributed additional anti-inflammatory benefit through its capacity to preserve and rebuild skeletal muscle mass, thereby reducing the systemic pro-inflammatory milieu associated with sarcopenia, which is prevalent and pathologically significant in COPD[12].

The superiority of supervised outpatient combined training over home-based aerobic exercise is a finding of direct clinical relevance. In Group B, while participants received weekly supervisory phone calls and standardized

written instructions, the absence of direct professional oversight, individualized load progression, and resistance training modalities appears to have limited the magnitude of physiological adaptation. This is congruent with evidence demonstrating that institute-based supervised pulmonary rehabilitation produces significantly greater functional and clinical improvements compared to conventional home-based exercise, a difference attributed to the precision of exercise prescription, real-time feedback, and the motivational environment of supervised sessions[11]. The inclusion of resistance training in a progressive, 1RM-guided protocol in Group A further distinguishes this intervention from standard home-based walking programs, as progressive overload which is difficult to implement safely without professional supervision is the principal driver of neuromuscular adaptation and antioxidant enzyme upregulation in skeletal muscle.[7] It has further been highlighted that structured, supervised non-pharmacological interventions in COPD produce improvements in exercise tolerance and systemic responses that home-based strategies frequently fail to replicate at equivalent intensity, underscoring the importance of the supervised setting as an independent therapeutic variable[15]. Evidence from concurrent exercise training in older COPD patients has also demonstrated reductions in plasma protein carbonyls of approximately 26.9% following combined supervised training compared to no change in controls, providing further mechanistic support for the oxidative benefits of combined supervised exercise observed in the current trial[17].

The sample size of the present study was derived from prior work reporting measurable changes in oxidative stress parameters following exercise in COPD patients, supporting the reliability of these biomarkers as sensitive indicators of training-induced biochemical adaptation[9]. Evidence from eccentric and concentric cycling protocols in stable COPD patients has further corroborated that multi-modal training modalities differentially affect pulmonary and plasma oxidative stress indices, with supervised protocols consistently producing larger effect sizes than unsupervised comparators[18]. Additionally, findings from endurance versus resistance training comparisons in COPD have demonstrated that resistance training uniquely upregulates skeletal muscle antioxidant enzyme expression, including SOD2, independent of the aerobic stimulus[19], which is consistent with the superior biochemical outcomes observed in Group A of the present trial. The current results further extend the current evidence of pulmonary rehabilitation by confirming that structured combined exercise, when delivered in accordance with ATS/ERS rehabilitation principles,[20,21] produces measurable and statistically significant improvements across pulmonary, oxidative, and inflammatory domains, concurrently, in a South Asian COPD population that remains underrepresented in the global rehabilitation literature.

The current study has a number of methodological strengths such as its randomized controlled design, blinded outcome assessment, allocation concealment, and the use of a multidomain outcome panel. However, there are some constraints that need to be recognized. The single-center design involving a tertiary academic institution can be limiting to the generalizability of the study to the community-based COPD populations with different disease severity profiles or access to healthcare. Even though the subjects in Group B were called once a week to take supervisory telephone calls, home exercise adherence could not be objectively measured in the absence of accelerometry or continuous heart rate monitoring, and self-reported compliance may be subject to social desirability bias. The 12-week follow-up period, even though it is consistent with the standard duration of pulmonary rehabilitation courses, does not permit making any conclusion about the sustainability of the observed biochemical and functional improvements beyond the active intervention phase. Moreover, no control or recording was done regarding the dietary intake of antioxidants which could independently regulate the levels of SOD and MDA. Multi-center trials with longer follow up periods, objective measures of adherence, dietary evaluation and dose response analyses are justified to further define the best exercise prescription to alter oxidative and inflammatory pathways in COPD.

CONCLUSION

The results of this randomized controlled trial show that 12 weeks of supervised combined aerobic and resistance training produces significantly more improvements in pulmonary function, oxidative stress markers and systemic inflammatory cytokines than home based

aerobic training alone in patients with COPD. The supervised combined protocol through its combination of progressive resistance training, individualized prescription of loads, and professional supervision had a superior and clinically significant therapeutic response in all outcome domains. These findings support the priority of supervised, multi-modal exercise training in the framework of pulmonary rehabilitation in patients with COPD and indicate the insufficiency of home-based aerobic exercise as a standalone intervention to achieve optimal biochemical and functional rehabilitation outcomes.

Author Contributions

MS: Conception & Design, Data Collection, Manuscript Writing, Final Approval
YKL: Data Analysis & Interpretation, Critical Revision
HBN: Data Collection, Data Analysis & Interpretation, and Final Approval
D: Data Analysis, Manuscript Writing, Final Approval
MH: Data Analysis, Manuscript Writing, Critical Revision and Final Approval
MN: Critical Revision and Final Approval

Conflict of Interests

No conflict of interest.

Data Availability

Data will be available upon request.

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No sources

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Prevalence of Skin and Gastrointestinal Infections Associated with Contaminated Water in Coastal Sindh: A Cross-Sectional Study

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ABSTRACT

Background: Water-borne diseases are still a significant preventable cause of morbidity in low-income coastal communities. The reliance on unprotected surface water sources is associated with gastrointestinal and skin infections in coastal areas of Sindh, Pakistan. The purpose of this study was to evaluate the prevalence of these infections and the relationship between them and drinking water sources and sanitation facilities for coastal populations.

Methods: It was a descriptive cross-sectional study conducted in coastal districts of Badin, Thatta and Sujawal, recruited 385 participants by convenience sampling. Questionnaire-based data were gathered on sociodemographic factors, water consumption habits, sanitation facilities and self-reported infections. Gastrointestinal symptoms that fulfilled the WHO criteria and dermatological symptoms from water contacts were defined as outcome measures. The data were analyzed by descriptive statistics and Chi-square test ($p<0.05$) through IBM SPSS version 26.

Results: 67.8% of participants reported gastrointestinal infections, with diarrhea being the most frequently reported symptom (48.8%). Skin infections were reported by 50.9%, mainly rashes and pruritus (34.3%). Gastrointestinal and skin infections were seen concurrently in 37.4% of the participants. There were significant relationships between gastrointestinal infections and the sources of primary drinking water ($\chi^2=32.84$, $p<0.001$) and drinking water source and skin infections ($\chi^2=22.61$, $p<0.001$), especially among users of canal or drain water.

Conclusion: The residents of coastal Sindh showed a high prevalence of gastrointestinal and skin infections, which were strongly linked to the use of unprotected water sources and poor sanitation. There is an urgent need to improve access to safe water, sanitation and promote hygiene.

Keywords: *Diarrhea, Gastrointestinal Diseases, Public Health, Skin Diseases, Water Borne Diseases*

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INTRODUCTION

Clean drinking water is a prerequisite for good health, but polluted water is one of the largest preventable causes of morbidity and mortality globally1. Gastrointestinal infections, including diarrhea, cholera, typhoid and dysentery are the most common water-borne diseases, causing millions of deaths every year, particularly in children under 5 years old in low and middle income countries[1,2]. In addition to enteric disease, quantitative microbial risk assessments have shown that pathogenic bacteria such as Escherichia coli, Salmonella spp., Shigella spp., Campylobacter, and Vibrio cholerae are widely distributed in drinking water sources and that they pose serious health risks to populations that are exposed, particularly in areas with inadequate water treatment and sanitation[3].

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It is evident that Pakistan is one of the most water stressed countries in the world and the quality of drinking water has been deteriorating consistently in the county due to the increasing population, rapid industrialization, agricultural runoff, and old sanitation system[2,4]. It is a fact that about 80% of all the diseases and nearly 40% of all deaths in the country are due to unsafe water consumption[4]. High prevalence of diarrhea, typhoid fever, dysentery and hepatitis, as well as cholera have been reported in several provinces such as Khyber Pakhtunkhwa and Punjab and are linked to contaminated water sources[5,6,7]. The challenge is further amplified during flooding events: following the catastrophic 2022 monsoon floods, over 1.5 million suspected cases of priority waterborne diseases were reported nationally, with Sindh Province alone contributing the highest burden approximately 692,673 cases across 23 districts.[8] District-level data from flood-affected areas of Khairpur Mirs documented a simultaneous surge in malaria, diarrhea, dysentery, cholera, typhoid, skin diseases, and hepatitis among affected communities, underscoring the multi-disease burden imposed by contaminated floodwater[9].

Sindh Province, particularly its coastal belt, represents a setting of compounded vulnerability. Coastal communities depend heavily on surface water from canals, tidal creeks, and open drains, many of which carry high microbial loads and chemical pollutants[10]. In addition to high microbial levels, groundwater in several coastal and riverine districts of Sindh has arsenic levels much higher than the limits set by the World Health Organization (WHO), thus also presenting a chemical health risk[10]. Microbiological risk assessment studies in Sindh found that approximately half of school water samples were contaminated with multiple pathogens and that southern Sindh carried the highest risk of infection, with children in Karachi exhibiting the greatest annual probability of illness due to *Campylobacter* (70%) and Rotavirus (22.6%)[1]. Poor water, sanitation, and hygiene (WASH) conditions have been independently associated with increased risk of gastrointestinal illness and skin disease in community settings[3,11], while rural drinking water assessments in similar resource-limited settings confirm that sanitation coverage and water treatment practices remain critically inadequate[11,12].

Despite the well-documented burden of waterborne disease in Pakistan, existing research has predominantly focused on enteric infections in non-coastal regions, with limited attention to the unique environmental, occupational, and sanitation conditions of coastal Sindh. The co-prevalence of skin infections and gastrointestinal illness attributable to contaminated water two disease categories known to co-occur in populations with heightened water contact remains largely uncharacterized in coastal Sindh[5,7]. There is therefore a pressing need for rigorous cross-sectional epidemiological evidence from coastal Sindh to quantify the prevalence of both skin and gastrointestinal infections associated with contaminated water exposure, identify associated socio-demographic and environmental risk factors, and generate data to inform targeted public health interventions.

METHODOLOGY

Study Design and Setting

This descriptive cross-sectional study was conducted across three coastal districts of Sindh, Pakistan: Badin, Thatta, and Sujawal. These districts were purposively selected on the basis of their coastal geography, documented dependence on unprotected water sources. The study was carried out over a period of twelve months, from October 2024 to September 2025.

Sample Size

The sample size was calculated using Open Epi version 3.0 software at a 95% confidence interval and a 5% margin of error. The reference prevalence was derived from Ali et al. (2023), which reported a prevalence of diarrhoea as 17% and skin diseases as 8% among flood-affected populations of district Khairpur Mirs, Sindh, Pakistan[9]. These two prevalence values were combined to obtain an estimated prevalence of 25%, which was used as the basis for sample size estimation. A final sample size of n = 289 participants was determined.

$$n = Z^2 \times p \times (1 - p) / d^2$$

Where $Z = 1.96$ (at 95% confidence), $p = 0.25$ (combined prevalence of gastrointestinal and skin infections), and $d = 0.05$ (margin of error). Participants were selected through non-probability convenience sampling from community health centers, basic health units (BHUs), and residential areas across the three study districts.

Selection Criteria

Residents aged 18 years and above who had been residing in the coastal districts of Badin, Thatta, and Sujawal for a minimum period of six months prior to the study and who used local water sources including canal or surface water, hand pumps, tube wells, or unpiped ground water for drinking or domestic purposes were included. Participants who gave written informed consent were enrolled. People who had been diagnosed with chronic non-water exposure dermatologic disease, such as psoriasis, congenital ichthyosis, had undergone gastrointestinal surgery, or had moved to the study area less than 6 months prior were excluded from the study.

Data Collection Procedure

Ethical approval was obtained from the Ethical Review Committee of DHQ Civil Hospital Thatta (IRB Protocol Number: DHQ-CH-012-24) and data collection was started after the approval. Written informed consent was taken from all participants before they were enrolled and the nature, voluntary nature and confidentiality of the study was explained to participants in both Sindhi and Urdu.

The trained field investigators administered a pre-tested questionnaire for data collection on the following variables: (i) sociodemographic characteristics such as age, gender, district of residence, educational level and monthly household income; (ii) primary drinking water source, and method of water storage; (iii) water treatment practices (boiled, filtered, chemically disinfected, or not treated); (iv) sanitation and hygiene practices (latrine access, handwashing behavior); and (v) history of gastrointestinal or skin infections in the previous 3 months.

Gastrointestinal infections were confirmed when a person reported having one or more of the following symptoms: diarrhea, vomiting, abdominal cramps, nausea or dysentery, according to the WHO case definitions for waterborne enteric illness. Skin infections were evaluated by self-reported rashes, skin sores, dermatitis, boils, and itching of the skin that occurred at the same time or immediately after being in water. An attempt was made to obtain clinical confirmation of disease episodes by using medical records or the Basic Health Units (BHUs) registers, if available, to support participant-reported disease episodes. The water sources observed and reported were classified into piped water supply, canal or surface water, hand pump or tube well, or bottled water.

Statistical Analysis

IBM SPSS Statistics 26 was used to enter and analyze the data. Categorical variables like demographic characteristics, type of water source, sanitation practices and disease prevalence were tabulated and presented as frequencies and percentages. The correlation between the type of water source, sanitation and incidences of skin and gastrointestinal infection was assessed by Chi-square test. A p value of < 0.05 was considered significant.

RESULTS

A total of 289 community residents from three coastal districts of Sindh Badin (n = 97, 33.6%), Thatta (n = 101, 35.0%), and Sujawal (n = 91, 31.5%) completed the study. Most participants were male (56.7%) with the age group of 31-45 years being the age group with the highest representation (40.5%). The participants were predominantly uneducated (60.9%) or had received only primary education (40.6%) while 44.3% had an income of less than PKR 20,000 per month in their household. Demographic and socioeconomic characteristics presented in Table 1.

Table 1. Demographic and Socioeconomic Characteristics of the Participants (n = 289)

Variable	Category	Frequency n (%)
Gender	Male	164 (56.7%)
	Female	125 (43.3%)
Age Group (years)	18–30	84 (29.1%)
	31–45	117 (40.5%)
	46–60	65 (22.5%)
	>60	23 (8.0%)

District	Badin	97 (33.6%)
	Thatta	101 (35.0%)
	Sujawal	91 (31.5%)
Education Level	No formal education	95 (32.9%)
	Primary level	81 (28.0%)
	Secondary level	73 (25.3%)
	Higher / graduate	40 (13.8%)
Monthly Household Income (PKR)	<20,000	128 (44.3%)
	20,000 – 40,000	107 (37.0%)
	>40,000	54 (18.7%)

Hand pumps and tube wells were the most commonly used primary drinking water source (45.3%), followed by canal or drain water (35.3%). The majority of participants (58.5%) consumed water without any form of treatment prior to use. Half of the participants stored water in open, uncovered containers (50.9%). Open defecation was reported by 37.4% of participants, and one-quarter (25.3%) rarely or never practiced handwashing with soap. Water source and sanitation characteristics was summarized in Table 2.

Table 2. Drinking Water Source, Treatment Practices, and Sanitation Conditions (n = 289)

Variable	Category	Frequency n (%)
Primary Drinking Water Source	Canal / drain water	102 (35.3%)
	Hand pump / tube well	131 (45.3%)
	Piped supply	38 (13.2%)
	Bottled water	18 (6.2%)
Water Treatment Practice	No treatment	169 (58.5%)
	Boiling	56 (19.4%)
	Filtration	43 (14.9%)
	Chemical disinfection	21 (7.3%)
Water Storage Method	Open containers	147 (50.9%)
	Covered containers	142 (49.1%)
Access to Latrine	Yes	181 (62.6%)
	No (open defecation)	108 (37.4%)
Handwashing with Soap	Always (before/after meals)	88 (30.4%)
	Sometimes	128 (44.3%)
	Rarely / Never	73 (25.3%)

The overall prevalence of gastrointestinal infections was 67.8% (n = 196). Diarrhea was the most frequently reported gastrointestinal condition (48.8%), followed by vomiting or nausea (30.1%), dysentery (23.9%), typhoid fever (11.8%), and cholera (6.2%). The overall prevalence of skin infections was 50.9% (n = 147). Among individual presentations, rashes or pruritus were most common (34.3%), followed by boils or furuncles (21.5%), contact dermatitis (18.7%), fungal skin infections (16.3%), and scabies (10.7%). Co-occurrence of both gastrointestinal and skin infections was observed in 37.4% (n = 108) of all participants, while 18.7% (n = 54) reported neither condition. Prevalence data detailed in Table 3.

Table 3. Prevalence of Gastrointestinal and Skin Infections among Study Participants (n = 289)		
Infection Category	**Specific Condition**	**Frequency n (%)**
Gastrointestinal Infections	**Overall prevalence**	**196 (67.8%)**
	Diarrhea	141 (48.8%)
	Vomiting / Nausea	87 (30.1%)
	Dysentery	69 (23.9%)
	Typhoid fever	34 (11.8%)
	Cholera	18 (6.2%)
Skin Infections	**Overall prevalence**	**147 (50.9%)**
	Rashes / Pruritus	99 (34.3%)
	Boils / Furuncles	62 (21.5%)
	Contact dermatitis	54 (18.7%)
	Fungal skin infection	47 (16.3%)
	Scabies	31 (10.7%)
Co-occurrence of GI + Skin	**—**	**108 (37.4%)**
Neither infection reported	**—**	**54 (18.7%)**

A statistically significant association was observed between the primary drinking water source and both gastrointestinal infections (χ^2 = 32.84, df = 3, $p < 0.001$) and skin infections (χ^2 = 22.61, df = 3, $p < 0.001$). Participants relying on canal or drain water reported the highest prevalence of both gastrointestinal infections (86.3%) and skin infections (69.6%). In contrast, participants using piped water supply and bottled water demonstrated substantially lower rates of both gastrointestinal infections (42.1% and 33.3%, respectively) and skin infections (31.6% and 38.9%, respectively). The results show that there is a distinct gradient in risk of infection based upon water source quality, with unprotected surface water posing the highest risk. Details given in Table 4.

Table 4. Association Between Primary Drinking Water Source and Gastrointestinal and Skin Infections (n = 289)

Water Source	GI Infected n (%)	Chi Square	p-value	Skin Infected n (%)	Chi Square	p-value
Canal / drain water	88 (86.3%)	*χ^2=32.84 (3),*	*p< 0.001*	71 (69.6%)	*χ^2=22.61*	*p< 0.001*
Hand pump / tube well	86 (65.6%)			57 (43.5%)		
Piped supply	16 (42.1%)			12 (31.6%)		
Bottled water	6 (33.3%)			7 (38.9%)		

DISCUSSION

In the present study, high prevalence of waterborne infection was found among the residents of three coastal districts of Sindh, the prevalence of gastrointestinal infection was 67.8%, skin infection was 50.9% and 37.4% of the participants had both skin and gastrointestinal infection. Such results are in line with the situation of gastrointestinal and infectious diseases found in Thatta district by the population-representative illness survey conducted by Malik et al.[13], which reported high rates of gastrointestinal and infectious diseases in a population with 75% adult illiteracy rate, 81% rural dwelling and restricted access to health care facilities, with thatta being ranked 22nd among 23 districts in the Human Development Index, Sindh.

These structural determinants are reflected in the demographic profile of our cohort, where 44.3% of participants were earning less than PKR 20,000 per month and more than 60% of the participants had only primary education or no education at all. The demographic profile of our cohort, accounted by 44.3% of people earning

less than PKR 20,000 per month and more than 60% of people having no education or primary education, is a plausible socioeconomic explanation for the persistently high infection burden observed in this coastal setting.

Hydrochemical evidence within the study area supports the statistically significant link between use of canal or drain water and gastrointestinal (86.3%) and skin infections (69.6%). In a thorough national water quality assessment, Kumar et al. recorded arsenic levels as high as 1,400 micrograms per litre (µg/L) in ground water from Sindh and found that bacteriological contamination caused by faecal coliforms was rampant in all the provincial water sources[14]. In the district of Sanghar, Sindh, a hydrochemical study revealed that 31.7% of the groundwater samples were unsuitable for drinking and 20% of groundwater samples showed high restriction for drinking purpose due to saline intrusion, suggesting multi-hazard contamination in tube well sources too[15]. A chemical study and multivariate analysis of groundwater in Sanghar district, Sindh also showed that water samples were contaminated by a number of hazardous substances such as chromium, lead, nickel, cadmium, and arsenic in significant proportion and it was concluded that the groundwater source is facing multi-hazard contamination in significant proportions[16]. These findings explain why hand pump and tube well users in our cohort still recorded substantially higher infection rates than piped or bottled water consumers, reflecting endemic multi-source contamination across coastal Sindh water infrastructure.

The skin infection prevalence of 50.9%, dominated by rashes or pruritus (34.3%), boils (21.5%), and contact dermatitis (18.7%), is consistent with the dermatological burden reported in Sindh during flooding events. Manzoor and Adesola documented approximately 143,870 confirmed skin disease cases at medical centers across southern Sindh during the 2022 monsoon floods, attributing them to direct contact with contaminated floodwater causing wound infections, dermatitis, and conjunctivitis[17]. Notably, our study was conducted outside acute flooding periods yet skin infection prevalence approached the magnitude observed during the 2022 disaster, suggesting that chronic exposure to contaminated canals and open drains generates a persistent dermatological disease burden in coastal Sindh largely independent of acute flood events. A comparative analysis by Faruqui and Khan in remote Sindh reinforced this finding, demonstrating markedly elevated rates of contact-transmitted and waterborne infections in post-flood phases and emphasizing contaminated water environments as ongoing breeding grounds for skin and enteric pathogens[18].

The inadequate WASH practices documented in this cohort are a major driver of the observed infection burden. Palo and Kanungo reported an adjusted odds ratio of 3.79 (95% CI: 1.23–11.70) for acute illness among rural participants consuming surface water compared to tube well water, in a population where approximately 40% practiced open defecation, closely paralleling the 37.4% open defecation rate and 25.3% inadequate handwashing rate in our cohort[19]. The finding that 58.5% of participants consumed untreated water and 50.9% stored water in open, uncovered containers further compounds post-collection contamination risk. These converging WASH deficits create the faecal-oral and contact transmission conditions that sustain both gastrointestinal and dermatological disease at the community level, consistent with the dual-burden pattern observed in our study population.

The high prevalence of diarrhea (48.8%), dysentery (23.9%), and typhoid fever (11.8%) in this cohort reflects the enteric pathogen exposure documented in Sindh water sources. The microbial risk assessment conducted by Ahmed et al. across ten districts of Sindh primary schools estimated a 70% annual probability of Campylobacter infection and a 22.6% annual probability of Rotavirus illness for children consuming school water in southern Sindh, driven by documented faecal coliform contamination in both groundwater and surface water sources[20]. In the present study, the same observations have been made in the context of the adult coastal community, which revealed that the level of pathogen exposure in the water source area in Sindh results in a significant level of enteric disease at the community level for all age groups in the coastal districts. In the bacteriological study of drinking water in Badin district by Ali et al., they also found that there is a significant presence of faecal coliforms in the water sources in the coastal belt which is in line with the enteric infection gradient observed across the different water sources in our cohort[21]. In this setting, gastrointestinal and skin infections occurred concurrently in 37.4% of participants,

highlighting multi-pathway modes of transmission of waterborne diseases. The communities having poor water and sanitation conditions are at higher risk of multiple co-occurring infectious diseases because water is a common source of transmission for various classes of infectious diseases[22], as it is observed that water sources are the common sources for the transmission of hepatitis B and hepatitis C virus. The co-occurrence pattern found in this cohort is thus a predictable consequence of chronic multi-route water exposure: enteric disease from ingestion of microbiologically contaminated water leads to dermatitis from contact with water contaminated by canals for domestic use, bathing, fishing and/or agricultural activities, and thus the observed dual burden pattern[23,25]. The climate-driven amplification of waterborne disease risk in coastal Sindh provides important context for interpreting the non-flood-period infection rates observed in this study. Faruqui and Khan demonstrated in remote Sindh communities that post-2022 flood phases corresponded to dramatic surges in malaria, hepatitis C, and other waterborne infections relative to pre-flood baselines, attributing these to floodwater mixing with sewage and agricultural drainage, contaminating canal and groundwater sources that communities continued to use for drinking and domestic purposes[18]. Manzoor and Adesola further documented that the 2022 floods displaced 2.3 million residents across Sindh and Balochistan and destroyed critical sanitation and water infrastructure, leaving a residual legacy of degraded source water quality that continues to elevate baseline infection risk in inter-flood periods[17]. The three coastal districts of this study, among the most severely flood-affected in Sindh, thus experience cyclical degradation of already fragile water infrastructure that compounds the chronic infection burden documented here.

There are a number of methodological weaknesses in this study that warrant mention. Disease ascertainment was performed by self-reported history of symptoms with the support of clinical record review if available, without routine laboratory confirmation of illness-causing pathogens, so that there is a potential for recall bias and misclassification of symptoms. The cross-sectional study design cannot establish causal association between water source exposure and occurrence of waterborne infections. Selection of recruited subjects from community health centers, basic health units, and residential areas could have also been a convenience sampling, which may have resulted in an overestimation of prevalence of the disease in comparison to the general population. Also, water quality testing at the household level is not done, which makes it difficult to directly attribute illness to exposure to any specific pathogen. A robust evidence base for causal attribution and targeted intervention design in this coastal population would be greatly enhanced by future longitudinal studies that included laboratory diagnosis and household water quality analysis and objective assessments of WASH infrastructure.

CONCLUSION

This cross sectional study shows a high clinically significant prevalence of gastrointestinal infection (67.8%) and skin infection (50.9%) among the residents of coastal Sindh, and more than one third of the respondents having a dual disease burden. The strongest determinants for both types of infection were primary drinking water source; canal and drain water consumers had a disproportionately high risk compared to piped or bottled water consumers. The lack of water treatment (58.5%) of water consumed, open defecation (37.4%) and poor hand hygiene (25.3%) places are a ‘convergent transmission environment' sustaining the observed infection burden regardless of acute flooding events. These results correspond to the regional hydrogeochemical evidence of widespread contamination of groundwater in coastal districts of Sindh and match with the national and international literature of the link between WASH deficits and waterborne disease burden in low-resource areas. The findings highlight the need of immediate public health interventions to increase access to safe piped water, improve sanitation access through increasing the coverage of latrine construction and hand washing promotion and implement community-level water treatment approaches (such as point of use chlorination, ceramic filtration) as an interim solution, in coastal Badin, Thatta and Sujawal districts. Coastal belt of Sindh should be regarded as a high-risk area of compounded infectious diseases that demands specific epidemiological surveillance, environmental health intervention as well as continuous investment in water, sanitation, and hygiene facilities for lowering the non-preventable morbidity burden as seen in this study.

Author Contributions
AS: Conception & design, Data Analysis and interpretation and Manuscript Writing.
NZ: Data Collection and Manuscript Writing.
AL: Conception & design, Data Collection, Critical Revision and Final Approval.
IK: Data Analysis and interpretation, Critical Review and Final Approval.

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Data will be available on request

Ethics approval
Not applicable

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Original Article

Apigenin and Carbenoxolone Co-Treatment Induces Potent Cytotoxicity in CAL-27 Oral Squamous Cell Carcinoma: A Morphological and Dose-Response Study

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ABSTRACT

Background: Oral squamous cell carcinoma is a major health burden across the globe due to its widespread incidence, limited effective therapeutics, and high recurrence rates. Natural and synthetic compounds such as Apigenin (API) and Carbenoxolone (CBX) have shown significant antitumor potential against various cancer types. The current study aimed to evaluate the anti-proliferative potential of API and CBX, alone or in combination in the CAL-27 oral cancer cell line.

Methods: MTT assay was used to analyze the cytotoxic potential of API, CBX, and their combination in the CAL-27 cell line. Differential Interference Contrast (DIC) microscopy was performed to identify alterations in cellular morphology after treatment.

Results: The half maximal inhibitory concentrations for API and CBX were calculated as 50 µM and 200 µM respectively. Significantly greater inhibition of 86.37% (p-value <0.001) was observed after combination treatment in the CAL-27 cell line. Microscopic examination revealed cell death, cell shrinkage and detachment, and membrane damage in combination-treated cells compared with control and single agent treatment groups.

Conclusion: Treatment with API and CBX exhibited decreased cell viability, and their combination further increased anti-proliferative effects in CAL-27 cells, suggesting that these compounds in combination are potential candidates for anticancer therapy in OSCC.

Keywords: *Apigenin, Carbenoxolone, Cytotoxicity, Combination therapy, Cytology, Oral squamous cell carcinoma.*

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INTRODUCTION

Oral Squamous Cell Carcinoma (OSCC) is one of the most prevalent malignancies of the oral cavity with significant impact on the global health burden, especially in the South Asia, where its prevalence continues to rise at a substantial rate.[1,2] Socioeconomic disparities, tobacco consumption, betel nut use, alcohol exposure, poor oral hygiene, poor nutritional status, and infection with tumorigenic HPV genotype strongly influence disease impact.[3-4]

Moreover, demographic transitions underscore a significant disparity and pronounced rise in OSCC within the male population, younger age groups, and underserved population, across intraoral regions, including buccal, labial mucosa, alveolar ridge, tongue, hard palate, retromolar trigone, and floor of the mouth.[5,6] Oral cancer has been shown to exhibit drug resistance towards various chemotherapeutic drugs, resulting in decreased treatment efficacy. Hence, there is a dire need to develop more effective and novel anticancer agents. While

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advancements in surgical and anticancer therapy continue, the treatment efficacy remains insufficient with undesired side effects, which stresses the necessity for developing newer therapies with optimized safety and efficacy profiles.

Bioactive phytochemicals have drawn interest as putative anticancer agents owing to their biodiversity and lower toxicity. Flavonoids comprise a key category of phytochemicals with anticancer properties.[7,8] Apigenin (API), a naturally derived flavonoid, has been extensively documented to attenuate cancer cell proliferation and enhance tumor cell death across diverse cancer types.[9] Its tumor-suppressive activity has been linked with restriction of cellular growth, attenuation of metastatic capacity, and induction of structural and morphological alterations in malignant cells by disrupting key signaling pathways such as PI3K/Akt, MAPK, and p53.[10]

Carbenoxolone (CBX), a derivative of glycyrrhetinic acid extracted from the liquorice plant and a gap junction inhibitor, is known to have a broad pharmacological profile, including anti-inflammatory, antiviral, anticancer, antibacterial, anti-fibrotic, and neuroprotective.[11,12] As proven from the previous studies that showed that CBX can decrease the cell viability by inducing apoptosis in multiple cancer cell lines.[13-15].

Although API and CBX have individually demonstrated anticancer activity in various malignancies, comparative studies examining their cytotoxic effects alongside morphological changes in OSCC cell lines are scarce. Combining both compounds can enhance their anticancer effect by simultaneously inhibiting proliferation and inducing cell death. Additionally, CBX-induced membrane and mitochondrial destabilization may sensitize cancer cells to API-mediated anti-proliferative signaling, resulting in synergistic or additive anticancer effects in OSCC. CAL-27 (tongue squamous cell carcinoma) cell line used in this study was selected because it is one of the most reported cell lines in the literature and is well-characterized at the molecular level. Compared to other oral cancer cell lines, it provides stable proliferation kinetics and reliable dose-response outcomes. CAL-27 exhibits an overactive PI3K/AKT pathway and is a suitable cell line for the investigation of cytotoxicity, proliferation, and migration.[16,17] Hence, the current study aimed to evaluate the cytotoxic effects of API and CBX in the OSCC (CAL-27) cell line using the MTT assay and to characterize treatment-induced morphological alterations via DIC microscopy.

METHODOLOGY

Cell Culture

The human oral squamous cell carcinoma cell line CAL-27 was used in this study. It was an in vitro experimental study conducted at Multidisciplinary Research Lab 1 (MDRL-1), Ziauddin University, Karachi, Pakistan. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Thermo Fisher Scientific, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were cultured at 37 °C in a humidified incubator with 5% CO_2 and sub-cultured at 80-90% confluency. Cells were treated with trypsin-EDTA. The cell line at passage number 7 was used for the study. The cell line was previously tested for mycoplasma contamination using DAPI staining and was found to be mycoplasma negative.

Reagents

API (A167323) and CBX (C4790) were obtained from Sigma- Aldrich (USA). Trypsin-EDTA (25200-056), Dulbecco's Modified Eagle Medium (11995-065), Foetal Bovine Serum (10270-106), Dimethyl Sulfoxide (137285925), MTT dye (M6494), Phosphate Buffered Saline (10010023), and Penicillin-Streptomycin (15070063) were obtained from Thermo Fisher Scientific (USA).

Cell Viability Assay

Cell viability was assessed using the MTT assay. CAL-27 cells were plated in 96-well plates at a density of 8×10^3 cells per well, and subjected to overnight incubation for cell

adherence. Different treatment concentrations of CBX (300 µM, 150 µM, 75 µM) and API (200 µM, 100 µM, 50 µM, 25 µM) were added. Combination treatment was performed using half maximal inhibitory concentrations of 50 µM for API and 200 µM for CBX for 48 hours. Following compound exposure, the MTT solution (0.5 mg/mL) was dispensed into each well and cultured for 3 h at 37°C to allow formation of formazan crystals. Removal of cell culture media was done, and crystals were solubilized in Dimethyl Sulfoxide (DMSO). To rule out vehicle (DMSO) induced cytotoxicity, vehicle control wells received the same amount of DMSO as the compound-treated wells. Furthermore, the final concentration of DMSO was ≤0.25% across all treatment groups, which is below the cytotoxic threshold for cell lines. Absorbance was determined at 570 nm using a MultiSkan Sky microplate reader. Relative cell viability was calculated using untreated control cells as a baseline. All experiments were conducted in triplicate and replicated independently three times.

Coefficient of Drug Interaction (CDI) for the half maximal inhibitory concentrations of 50 µM for API and 200 µM for CBX was determined by using fractional viability using the percent inhibition formula:

- **Fractional Viability** = 1 − (Cell Death (%) / 100)

- **CDI** = Combination of Drug A & B / Drug A x Drug B

Differential Interference Contrast (DIC) Microscopy

The cells were plated at a density of 50,000 cells per well in a 24-well plate. Cells were incubated for 24 hours before treatment to induce morphological changes. Afterwards, the compounds API, CBX, and their co-treatment were introduced for 48 hours. Post treatment, cells were rinsed with PBS, and DIC microscopy was used to assess and document morphological changes. Imaging was done using a Nikon Eclipse Ts2 microscope at 10x magnification using Nikon NIS-Elements imaging software.

Statistical Analysis

Analysis was done using SPSS version 24.0. The graphs were made using the same software. Percent inhibition in the control, API, CBX, and co-treatment groups was expressed as mean ± standard deviation. Normality of the data distribution was assessed using the Shapiro-Wilk test. For comparison among multiple groups, one-way analysis of variance (ANOVA) was applied, followed by Tukey's post hoc test for pairwise comparison, along with 95% confidence interval (CI) for the estimation of the magnitude and precision of the treatment effects. A p-value of less than 0.05 was considered significant.

RESULTS

API and CBX Reduce CAL-27 Cell Viability in a Dose-Dependent Manner

Viability of CAL-27 cells following treatment with API and CBX was evaluated using the MTT assay. Both compounds exhibited a reduction in cell viability in a dose-dependent manner as compared to untreated (DMSO vehicle control) CAL-27 cells.

Treatment with API resulted in growth inhibition of approximately 11.52±3.99 (95%CI=1.58-21.45), 51.16±1.850 (95%CI=46.57-55.76), 69.23±1.950 (95%CI= 64.38-74.07), and 70.48±2.17 (95%CI= 65.08-75.88) at 25 µM, 50 µM, 100 µM, and 200 µM, respectively, after 48 hours (Figure 1A). These doses and times were selected after a literature review and a pilot MTT assay. Approximately 50% inhibition of cell viability was observed at 50 µM, indicating the half-maximal inhibitory concentration of API in CAL-27 cells.

CBX treatment also induced a dose-dependent reduction in cell viability. Growth inhibition values of approximately 3.1652±2.20 (95%CI=-2.31-8.65), 10.39566±0.52, (95%CI= 9.09-11.69), and 80.6341±3.60, (95%CI= 71.69-89.57) were observed at 75 µM, 150 µM, and

300 µM (Figure 1B). Concentrations below 75 µM did not inhibit the cell growth, and were therefore not reported. The estimated half-maximal inhibitory concentration of CBX was 200µM. To evaluate potential combined effects, CAL-27 cells were co-treated with API (50 µM) and CBX (200 µM). This combination treatment produced the greater reduction in cell viability of 86.37±1.40 (95%CI= 82.87-89.85), exceeding the inhibitory effects observed with individual treatments (Figure 1C). The combination of API and CBX half maximal inhibitory concentration showed synergistic/enhanced effect after calculation (Table 1). The statistical analysis for inter and intra group comparisons and multiple comparisons between compounds are depicted in Table 2 and 3.

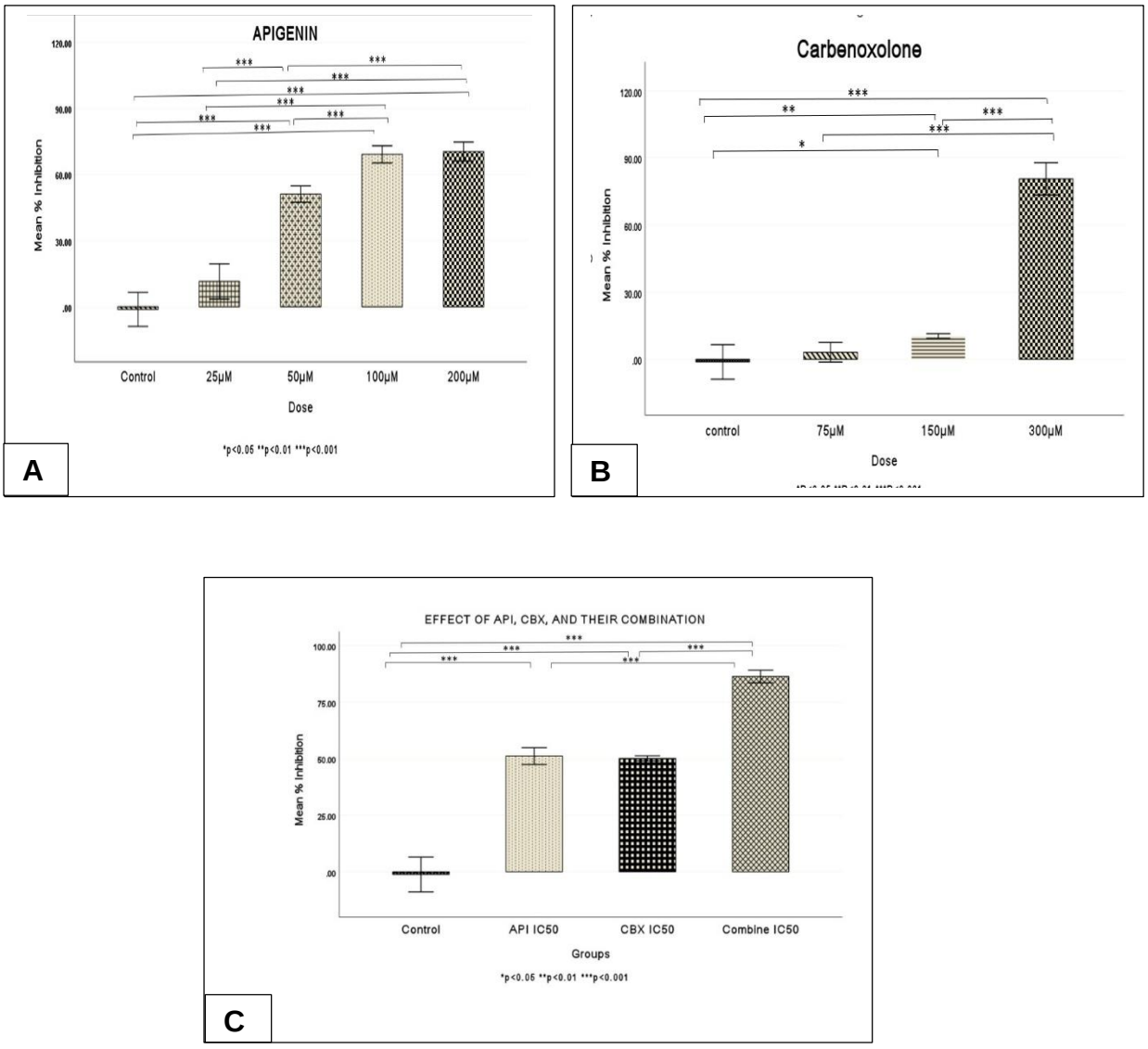

Figure 1: Dose-dependent cytotoxicity effects of Carbenoxolone, Apigenin, and their combination in CAL-27 cells (A)percentage inhibition at varying API concentrations (B)percentage inhibition at varying CBX concentrations, (C) percentage inhibition at the half maximal inhibitory concentration of API, CBX, and their combination, with maximal inhibition observed at combine half maximal inhibitory concentration. Data are presented as mean ± SD of three independent experiments. Statistical analysis was performed using One-way ANOVA followed by Tukey's post hoc multiple comparison test. Differences compared among various groups were observed at $p < 0.05$, $p < 0.01$, and $p< 0.001$.

Table-1: Coefficient of Drug Interaction (CDI) Analysis Using Fractional Viability

Combination Dose	% Cell Death	% Cell Viability	Fractional Viability	CDI Values	Interpretation
CBX 200µM	50%	50%	0.50	0.558	Synergistic Effect
API 50µM	51.16%	48.84%	48.84		

Table-2 One-Way ANOVA Analysis of Apigenin and Carbenoxolone-Induced Inhibition

ANOVA (APIGENIN)			
	df	**F-statistic**	**Significance**
Between Groups	4		
Within Groups	10	386.626374	<.001
Total	14		
ANOVA (Carbenoxolone)			
	df	**F-statistic**	**Sig.**
Between Groups	3		
Within Groups	8	540.801070	<.001
Total	11		

Table-3 Multiple Comparison for Apigenin and Carbenoxolone (Post Hoc Tukey Test)

	Group-wise Comparison		**Significance**
Apigenin	**Control**	**API 25 µM**	0.002
		API 50 µM	<.001
		API 100 µM	<.001
		API 200 µM	<.001
	25 µM	**API 50 µM**	<.001
		API 100 µM	<.001
		API 200 µM	<.001
	50 µM	**API 100 µM**	<.001
		API 200 µM	<.001
Carbenoxolone	**Control**	**CBX 150 µM**	0.005
		CBX 300 µM	<.001
	75 µM	**CBX 300 µM**	<.001
	150 µM	**CBX 300 µM**	<.001

Morphological Alterations Observed by DIC Microscopy

DIC microscopy was used to evaluate the significant morphological changes in the CAL-27 cell line, which showed a notable difference between the treated and untreated groups. In the untreated group (control), the cells displayed typical epithelial morphology, with adherent cells and cellular contact, all in a healthy, proliferative condition without any cellular stress (Figure 2A). On the other hand, the API-treated group showed enhanced morphological changes, indicating decreased cellular health, including early signs of cell death, cell rounding, cellular shrinkage, cytoplasmic condensation, membrane disruption, and cell detachment from the cell culture surface (Figure 2B).

Likewise, CBX-treated cells showed marked cellular changes (Figure 2C). Signs of cellular stress, including cell rounding, irregular cell boundaries, reduced cell count, and loss of adhesion to the cell culture surface, indicate cell death. Remarkably, combined treatment with API and CBX (Figure 2D) appeared to cause the most pronounced morphological damage among all groups. The cells showed extensive damage to cell membranes, loss of adherence, and pronounced cytoplasmic shrinkage. Cellular fragmentation and lysis were also observed post-treatment, indicating cell death. All treatment groups exhibited cytotoxic effects, however the most notable decrease in cell viability and structural integrity was observed in the combination treatment group as compared to the control.

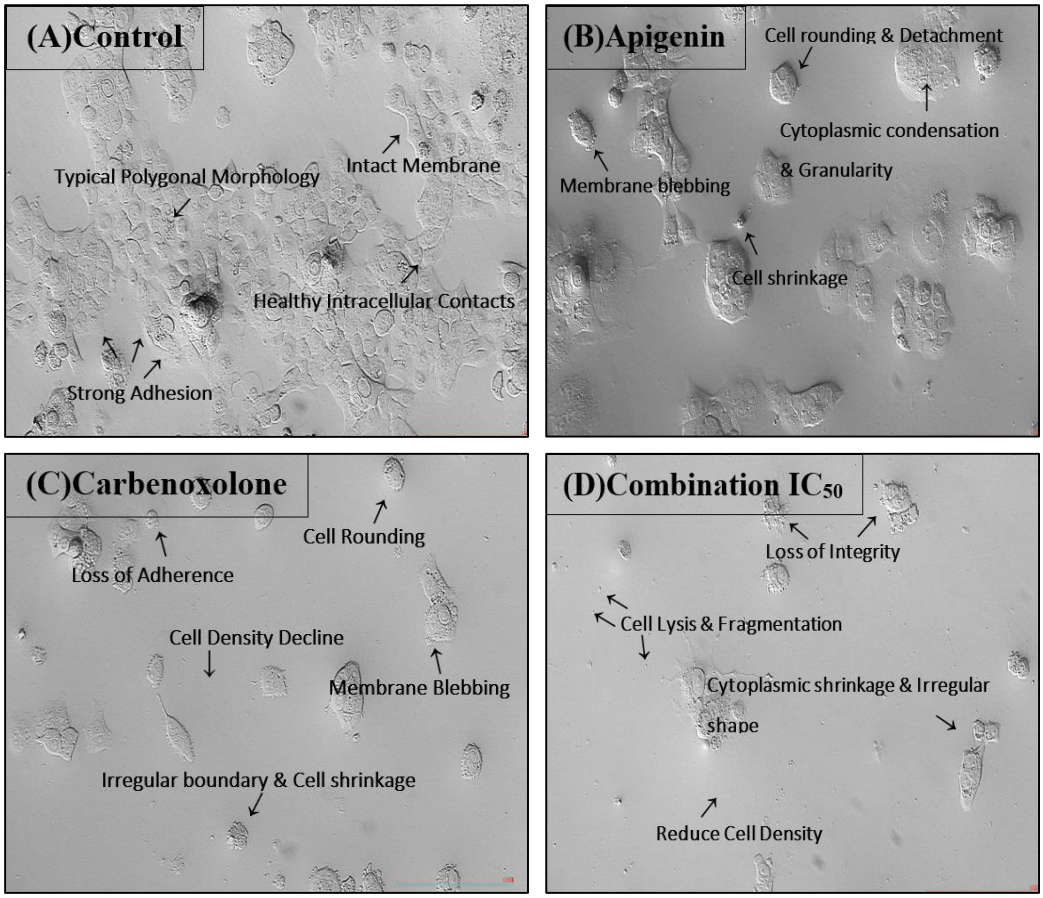

Figure 2: CAL-27 cells after treatment for 48 hours as observed by differential interference contrast (DIC) microscopy. Cells treated with API, CBX, and their combination displayed cytotoxic effects, scale bar 50µm.

DISCUSSION

API is widely considered a natural compound and a flavonoid widely considered as anticancer. The research findings of this study are consistent with results reported in the literature showing a dose-dependent effect. Decreased HSC-3 tongue carcinoma cell line proliferation following API exposure demonstrated its capacity to inhibit tumor cell growth even at comparatively low concentrations. In gastric carcinoma cell lines, API was found to decrease cell viability via dysregulating cell survival pathways and induction of apoptosis. The similar inhibitory pattern seen in this study is aligned with the broad anticancer activity of API against epithelial cancers.[18]

Comparatively lower sensitivity was observed in the CBX group against the CAL-27 cell line. Similar findings were also observed against K562 leukemia cells, where the compound induced proliferation reduction at around 150µM concentration. The comparatively higher half-maximal inhibitory concentration observed in the CBX group in this study may reflect tumor-specific responses toward drug treatment, influenced by cancer type and molecular background.[19] In another study, CBX exhibited anticancer effects against small cell lung carcinoma (SCLC) cells at concentrations ranging from 0-100µM by targeting pannexin 1, which in turn suppressed the extracellular ATP levels.[20]

The combination therapy showed enhanced efficacy compared with individual groups, indicating that combination strategies targeting complementary cellular pathways are effective approaches in cancer therapy. API and CBX target complementary oncogenic pathways and cause enhanced cell death. As per evidence that, API targets the cancer cell survival pathways such as PI3K/AKT, and CBX acts at the intercellular level by inhibiting gap junctions, simultaneously targeting intracellular survival pathways and intercellular communication, and metabolic pathways can produce a multi-targeted anticancer effect.[21,22]

Despite the antitumor potential of API and CBX, their efficacy across different cancers varies. For instance, an API's ability to induce apoptosis can vary depending on signaling pathway modulations within specific tumors.[23] Similarly, the differences in sensitivity observed with CBX at high concentrations in certain suspension cancer cell lines, compared with lower sensitivity in solid tumors, highlight tumor-specific effects. CBX can also function as a channel blocker, suppressing cancer proliferation.[24]

Morphological evaluation further supported the cytotoxic findings. Untreated CAL-27 cells maintained epithelial morphology, whereas treated cells displayed structural alterations consistent with cellular stress and death. Similar morphological changes following API treatment have been reported in endometrial carcinoma cells, in which cell shrinkage, loss of adhesion, and reduced spreading were associated with apoptotic processes.[25] The Same morphological alteration was seen in the leukemia model after treatment of the leukemic cells with CBX[19]. In the combination treatment group, the most significant structural damage was seen which intensified cellular damage. Therefore, supporting the findings of reduced cell viability in the MTT assay.

The selective toxicity is crucial for the creation of anticancer drugs. The prior experimental evidence supports API's minimal cytotoxicity in non-malignant cellular systems, and it was seen in the retina of rats both in vivo and in vitro.[26] Similar effects of CBX have been reported to modulate intracellular signaling in normal endothelial cells without inducing irreversible cytotoxic damage.[27] These findings show the potential therapeutic relevance of API and CBX as supplementary anti-tumor candidates with acceptable safety profiles, although confirmation in OSCC-specific normal cell models remains necessary.

Collective findings of the present study suggest that API functions as a potent growth inhibitor in OSCC cells, while CBX contributes additional cytotoxic stress that enhances overall treatment

efficacy when used in combination. Such multi-agent strategies may represent a promising approach for improving therapeutic responses in oral cancer while minimizing drug-associated cytotoxicity.

Limitations

The use of a single OSCC cell line may not fully reflect the effect of compounds, and results may not apply to other oral cancer subtypes. Furthermore, the study lacks critical molecular analysis, such as apoptotic evaluation, ROS production studies, and gene expression analysis. These parameters could have provided a deeper understanding of the molecular mechanism of action of these compounds. Furthermore, in vivo animal studies were also not conducted.

CONCLUSION

This study highlights the significant anticancer effects of API and CBX in the CAL-27, a representative cell line of OSCC. API exhibited higher potency as compared to CBX, and their combination resulted in enhanced anti-proliferative effects. Combination treatment also resulted in structural alterations as well as significant cell death, as evident by microscopic examination. These findings support the combined therapeutic potential of API and CBX in OSCC.

Author Contributions

AM: Conception & Design, Data Collection, Data Analysis & Interpretation, Manuscript Writing, Critical Revision and Final Approval
RI: Conception & Design, Data Collection, Data Analysis & Interpretation, Critical Revision and Final Approval
SH: Critical Revision and Final Approval
AB: Critical Revision and Final Approval
AH: Data Analysis & Interpretation, Critical Revision and Final Approval

Ethical Approval

This study is approved by the Ethical Review Committee of Ziauddin University, Karachi, Pakistan **(Ref: 7740923ABPHY)**

Conflict of Interests

The authors proclaim no potential bias.

Data Availability

Data will be available upon request.

Funding Source

No sources.

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Short Communication

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Knowledge, Attitude and Practice on Awareness of Pharmaceutical Waste Disposal among Local Public of Karachi

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ABSTRACT

Inappropriate disposal of expired and unused drugs by households is a major source of environmental pollution and contamination, posing a significant health hazard. This study aims to evaluate the general knowledge and practices of pharmaceutical waste disposal among the masses of Karachi. The study is a descriptive cross-sectional survey involving household members who use prescribed and Over the counter (OTC) medicines from different localities of Karachi. The study was conducted through a predesigned structured questionnaire from January 2025 to March 2025. A total of 2000 participants were included. Results revealed that 74% participants were aware of the harmful effects of medicinal wastes. However, only 4% were following drug take back programs, 96% even educated participants were oblivious to the existence of any such system. Around 86% of the participants mentioned disposing of expired/unused medicines in the dustbin, while 8% admitted flushing them down the sink/toilet. Hence, it is concluded that knowledge and proper implementation of pharmaceutical waste disposal are severely lacking in this global city and require urgent attention to prevent further adverse effects on human health.

Keywords: *Drug take back program, Human health, Pharmaceutical waste disposal.*

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INTRODUCTION

Over the past few decades, social, commercial, and lifestyle alterations have increased the occurrence of ailments, including obesity, diabetes, cardiovascular diseases, tumors, and autoimmune diseases, which in turn has accelerated the consumption of pharmaceuticals[1]. Karachi, the largest city in Pakistan, with an estimated population of 20 million, generates over 16500 tons of municipal waste daily[2]. Pharmaceutical waste includes expired or unused pharmaceutical products, spilled or surplus drugs, vaccines, or sera, and discarded bottles, boxes, gloves, masks, tubes, vials, blades, broken glass, and radioactive wastes[3].

In developing countries, self-medication, polypharmacy, and lack of knowledge are the factors leading to inaccurate disposal of pharmaceuticals[4]. Inappropriate disposal, for example, in dustbins or on road sides, causes serious health hazards such as respiratory distress, contamination of drinking water, accidental poisoning, accumulation in food chains to the general population[5] and leads to the evolution of antibiotic-resistant bacteria[6]. Major hurdles in the management of pharmaceutical waste include insufficient storage space, insufficient waste transportation facilities, inadequate and improper disposal sites, and the high cost of processing the waste. This study aims to evaluate the existing knowledge and practices of pharmaceutical waste disposal among the masses of Karachi.

MATERIAL and METHODS

This was a cross-sectional, community-based study conducted from January 2025 to March 2025 approved from department of Pharmaceutical Chemistry, Institute of Pharmaceutical Sciences, Jinnah Sindh Medical

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University (Ref No: IPS/DRC/PCM/2024/15). Data was collected from randomly selected 2000 respondents (aged 18-60 years) from different localities of Karachi using a structured, closed-ended questionnaire in English.

The questionnaire included demographic details, questions related to knowledge, attitude, and practice about the use and disposal of medicines. The terminologies were thoroughly explained to participants, where needed. Participants who were unable to understand English were excluded. An informed consent was taken from participants priorly.

RESULT

The data collected from common household people aged between 18 to 60 years, where 72% of the respondents were female and 28% were male. The results revealed that 79.02% respondents keep the medicines at home for later use, until expired. Around 74% participants were aware of the harmful effects of medicinal wastes but only 4% were returning the unused medicines to community pharmacies and were aware of drug take back programs. Almost 96% were oblivious to the existence of any such system. Around 86% of the participants mentioned disposing of expired/unused medicines in the dustbin, while 8% admitted flushing them down the sink/toilet. 95% of the respondents consider government authorities, physicians and pharmacist are equally responsible to propagate awareness on drug disposal through social and print media and 99% agreed to include such knowledge in educational system.

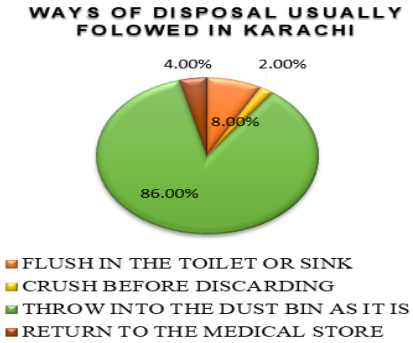

Fig. 1 Ways of disposal of pharmaceutical wastes in Karachi

DISCUSSION

In economically underdeveloped countries, the major cause of inaccurate disposal of pharmaceutical wastes is actually the lack of proper guidelines[4]. The results recorded in the present survey revealed that Karachi is severely lacking in knowledge and practices of proper pharmaceutical waste disposal. Majority, even the educated people, were unaware of the proper disposal methods. According to the data collected 99% of the respondents were keeping the unused medicines at home for future use which was a major reason for self-medication and poly pharmacy practices which give rise to excessive pharmaceutical waste. Results shown in figure 1 indicated that chief method for disposal, used by local people, was throwing in dust bins followed by flushing in toilet or sink which coincides with results in the other studies. In a review study Tabassum et al., reported house hold trash as the major way of disposal followed by flushing and limited percentage of return to pharmacies in Asian countries[8].

The study found that 74 % of people were aware of the adverse effects of pharmaceutical wastes however 10% respondents were absolutely unaware of any such effects and 16% were not sure. Similarly a study declared majority of the participants were aware of risks of improper pharmaceutical waste disposal still discarded wastes in house hold bins[9]. This is due to inadequate information about the discarding methods. In this respect, the following risk minimization measures can be adopted organizing awareness campaigns for masses, to provide information related to the use and the return of medicinal products, using clear labels that specify proper use and disposal, developing regulations for internet pharmacies and implementation of laws devised for proper disposal of pharmaceutical wastes. However the study explored the situation in Karachi only, further studies are required to evaluate the issue all over the country.

CONCLUSION

It was concluded that knowledge of proper pharmaceutical waste disposal is severely lacking in this global city and must be addressed urgently. The successful implementation of an accurate waste-disposal system requires proper legislation, the engagement of all stakeholders in educating the masses, and the provision of appropriate sites and technology for disposal.

Author Contributions

EZ: Data Collection and Manuscript Writing
KR: Conception & Design, Final Approval
SU: Data Analysis, Critical Revision
BH: Data Collection

Conflict of Interests

No conflict of interest.

Data Availability:

Data will be available upon request.

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No sources

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